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(54) DNA ENCODING A HUMAN NEUROPEPTIDE Y/PEPTIDE YY/PANCREATIC POLYPEPTIDE RECEPTOR (Y4) AND USES THEREOF

DNA CODIEREND FÜR EINEN MENSCHLICHEN NEUROPEPTID Y/PEPTID YY/PANKREASPOLYPEPTID-REZEPTOR (Y4) UND SEINE VERWENDUNG

ADN CODANT UN RECEPTEUR (Y4) DE NEUROPEPTIDE Y/PEPTIDE YY/POLYPEPTIDE PANCREATIQUE ET UTILISATION DE CELUI-CI

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EP 0 746 332 B1

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Description

[0001] Throughout this application, various publications are referenced in parenthesis by Author and year. Full citations for these references may be found at the end of the specification immediately preceding the claims. The disclosure of these publications is hereby incorporated by reference into this application to describe more fully the art to which this invention pertains.

[0002] Neuropeptides are small peptides originating from large precursor proteins synthesized by peptidergic neurons and endocrine/paracrine cells. They hold promise for treatment of neurological, psychiatric, and endocrine disorders (De Wied, 1990). Often the precursors contain multiple biologically active peptides. There is great diversity of neuropeptides in the brain caused by alternative splicing of primary gene transcripts and differential precursor processing. The neuropeptide receptors serve to discriminate between ligands and to activate the appropriate signals. Thus, it is expected that the receptors for neuropeptides consist of a large number of members.

[0003] Neuropeptide Y (NPY), a 36-amino acid peptide, is the most abundant neuropeptide to be identified in mammalian brain. NPY is an important regulator in both the central and peripheral nervous systems (Heilig et al., 1990) and influences a diverse range of physiological parameters, including effects on psychomotor activity, food intake, central endocrine secretion, and vasoactivity in the cardiovascular system. High concentrations of NPY are found in the sympathetic nerves supplying the coronary, cerebral, and renal vasculature and has contributed to vasoconstriction. NPY binding sites have been identified in a variety of tissues, including spleen (Lundberg et al., 1988), intestinal membranes, brain (Hinson et al., 1988), aortic smooth muscle (Mihara at al., 1989), kidney, testis, and placenta (Dumont et al., 1992). In addition, binding sites have been reported in a number of rat and human cell lines (eg. Y1 in SK-N-MC, MC-IXC, CHP-212, and PC12 cells; Y2 in SK-N-Be(2), CHP-234, and SMS-MSN)(Aakerlund et al., 1990; Grundemar et al., 1993).

[0004] NPY forms a family (called the pancreatic polypeptide family) together with pancreatic polypeptide (PP) and peptide YY (PYY) which all consist of 36 amino acids and have a common tertiary structure, the so-called PP-fold (Glover et al., 1985). Specific features of this family include a polyproline helix in residues 1 through 8, a β-turn in residues 9 through 14, an α-helix in residues 15 through 30, an outward-projecting C-terminus in residues 30 through 36, and a carboxy terminal amide which appears to be critical for biological activity (Schwartz et al., 1990). The C-terminal amidated residue of these peptides is essential for biological activity (Wahlestedt et al., 1986). Studies with peptide fragments of NPY have indicated that multiple NPY receptor subtypes exist (Wahlestedt et al., 1986). Three major NPY receptor subtypes (Y1, Y2 and Y3) have been defined by pharmacological criteria, with a fourth "atypical" Y1 receptor that has been proposed to regulate feeding behavior. The only NPY receptor which has been cloned to date is the Y1 receptor gene, from mouse (Eva et al., 1992), rat (Eva et al., 1990), and human (Larhammar et al., 1992). One of the key pharmacological features which distinguish Y1 and Y2 is the fact that the Y1 receptor (and not the Y2 receptor) responds to an analog of NPY modified at residues 31 and 34 ([Leu31,Pro34]NPY), whereas the Y2 receptor (and not the Y1 receptor) has high affinity for the NPY peptide carboxyl-terminal fragment NPY-(13-36)(Wahlstedt et al., 1986; Fuhlendorff et al., 1990).

[0005] Receptor genes for the other two structurally related peptides, peptide YY (PYY) and pancreatic polypeptide (PP), also have not been cloned. Peptide YY occurs mainly in endocrine cells in the lower gastrointestinal tract (Bottcher et al., 1984). Receptors for PYY were first described in the rat small intestine (Laburthe et al., 1986). This receptor has been defined as PYY-preferring because it displays a 5-10 fold higher affinity for PYY than for NPY (Laburthe et al., 1986; Laburthe, 1990). Recently, a cell line, PKSV-PCT, derived from the proximal tubules of kidneys, has been described to express receptors for PYY (Voisin et al., 1993). Pancreatic polypeptide is predominantly located in endocrine cells of the pancreatic islets (Alumets et al., 1978). PP inhibits pancreatic exocrine secretion and gall bladder contraction (Schwartz, 1983). Interestingly, PP does not appear to be synthesized in or localized to the central nervous system (Di Maggio et al., 1985), but selective PP binding sites have been found in various brain areas, such as the area postrema and adjacent nuclei, regions permeable at the blood-brain barrier (Whitcomb et al., 1990). PP receptors have a much higher affinity for PP than for NPY or PYY (Inui et al., 1990). PP has been shown to bind with high affinity to binding sites on a pheochromocytoma cell line, PC12 (Schwartz et al., 1987). The rank order of affinity for the pharmacologically defined receptors of NPY and related peptides are listed in Table 1.

[0006] Using an homology screening approach to clone novel NPY receptor genes, we describe here the isolation and characterization of a novel NPY/PYY/PP receptor clone which we have designated Y4. The Y4 receptor appears to have a unique pharmacological profile, relative to other NPY-related receptors, exhibiting highest affinity for pancreatic polypeptide itself. This receptor clone will enable us to further examine the possibility of receptor diversity and the existence of multiple subtypes within this family of receptors. These could then serve as invaluable tools for drug design for several pathophysiological conditions such as memory loss, depression, anxiety, epilepsy, pain, hypertension, locomotor problems, circadian rhythm disorders, eating/body weight disorders, sexual/reprductive disorders, nasal congestion, diarrhea, gastrointestinal and cardiovascular disorders.

[0007] This invention provides an isolated nucleic acid molecule encoding a human Y4 receptor, i.e., a receptor char-

acterized by a pharmacological profile characteristic of the human Y4 receptor as shown in Table 6.

[0008] This invention also provides an isolated nucleic acid molecule encoding a rat Y4 receptor, for example a receptor characterized by a pharmacological profile characteristic of the rat Y4 receptor as shown in Table 6.

[0009] This invention also provides an isolated protein which is a human or rat Y4 receptor and is encoded by the nucleic acid molecules as mentioned above.

[0010] This invention provides a vector comprising the isolated nucleic acid molecules encoding a Y4 receptor as mentioned above.

[0011] This invention also provides vectors such as plasmids and baculovirus comprising the nucleic acid molecules encoding a Y4 receptor as mentioned above, adapted for expression in a bacterial cell, a yeast cell, an insect cell or a mammalian cell which additionally comprise the regulatory elements necessary for expression of the nucleic acid in the bacterial, yeast, insect or mammalian cells operatively linked to the nucleic acid encoding the Y4 receptor as to permit expression thereof.

[0012] This invention provides a mammalian cell comprising the nucleic acid molecules encoding a Y4 receptor as mentioned above.

[0013] This invention provides a method for determining whether a ligand can specifically bind to a human or rat Y4 receptor which comprises contacting a plurality of cells transfected with and expressing the nucleic acid encoding a Y4 receptor as mentioned above with the ligand under conditions permitting binding of ligands to such Y4 receptor, and detecting the presence of any of the ligand bound to a Y4 receptor, thereby determining whether the ligand binds specifically to a human or rat Y4 receptor.

[0014] This invention also provides a method for determining whether a ligand is a human or rat Y4 receptor agonist which comprises contacting a plurality of cells transfected with and expressing the nucleic acid encoding a Y4 receptor as mentioned above with the ligand under conditions permitting the activation of a Y4 receptor functional response from the cells, and detecting by means of a bioassay, such as a second messenger response, an increase in Y4 receptor activity thereby determining whether the ligand is a human or rat Y4 receptor agonist.

[0015] This invention further provides a method for determining whether a ligand is a human or rat Y4 receptor antagonist which comprises contacting a plurality of cells transfected with and expressing the nucleic acid encoding a Y4 receptor as mentioned above with the ligand trader conditions permitting the activation of a functional Y4 receptor response, and detecting by means of a bioassay, such as a second messenger response, a decrease in Y4 receptor activity, and thereby determining whether the ligand is a human or rat Y4 receptor antagonist.

[0016] This invention further provides a method of screening chemical compounds to identify drug candidates which specifically bind to a human or rat Y4 receptor which comprises contacting a plurality of cells transfected with and expressing the nucleic acid encoding a Y4 receptor as mentioned above with a plurality of compounds, and determining those compounds which bind to the cell, thereby identifying drug candidates which specifically bind to a human or rat Y4 receptor.

[0017] This invention also provides a method of screening chemical compounds to identify drug candidates which act as agonists of a human or rat Y4 receptor which comprises contacting a plurality of cells transfected with and expressing the nucleic acid encoding a Y4 receptor as mentioned above with a plurality of compounds under conditions permitting the activation of a functional Y4 receptor response, and determining those compounds which activate the receptor in the cell using a bioassay, such as a second messenger assay, thereby identifying drug candidates which act as human or rat Y4 receptor agonists.

[0018] This invention also provides a method of screening chemical compounds to identify drug candidates which act as antagonists of a human or rat Y4 receptor which comprises contacting a plurality of cells transfected with and expressing the nucleic acid encoding a Y4 receptor as mentioned above with a plurality of compounds in the presence of a known human Y4 receptor agonist under conditions permitting the activation of a functional Y4 receptor response and determining those compounds which inhibit the activation of the receptor in the cell using a bioassay such as a second messenger assay, thereby identifying drug candidates which

[0019] act as antagonists of a human or rat Y4 receptor. This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of the nucleic acid molecule encoding a Y4 receptor as mentioned above.

[0020] This invention also provides a method of detecting expression of human or rat Y4 receptor on the surface of a cell by detecting the presence of mRNA coding for said Y4 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the nucleic acid probe as mentioned above comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding the Y4 receptor under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the human or rat Y4 receptor by the cell.

[0021] This invention provides an antisense oligonucleotide having a sequence capable of hybridizing specifically to an mRNA molecule which encodes a Y4 receptor as mentioned above so as to prevent translation of the mRNA molecule.

[0022] This invention provides an antibody directed to a human or rat Y4 receptor encoded by the nucleic acid molecule as mentioned above.

[0023] This invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific human Y4 receptor allele which comprises: a. obtaining nucleic acid of subjects suffering from the disorder; b. performing a restriction digest of the nucleic acid with a panel of restriction enzymes; c. electrophoretically separating the resulting nucleic acid fragments on a sizing gel; d. contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to nucleic acid encoding the human Y4 receptor and labelled with a detectable marker; e. detecting labelled bands which have hybridized to the nucleic acid encoding the human Y4 receptor labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f. preparing nucleic acid obtained for diagnosis by steps a-e; and g. comparing the unique band pattern specific to the nucleic acid of subjects suffering from the disorder from step e and the nucleic acid obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

[0024] This invention provides a method of preparing the purified, isolated human or rat Y4 receptor encoded by the nucleic acid as mentioned above which comprises a) constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of nucleic acid in the cell operativley linked to the nucleic acid encoding a Y4 receptor as to permit expression thereof, wherein the cell is selected from the group consisiting of bacterial cells, yeast cells, insect cells and mammalian cells; b) inserting the vector of stepa in a suitable host cell; c) incubating the cells of step b under conditions allowing the expression of a Y4 receptor; d) recovering the receptor so produced; and e) purifying the receptor so recovered, thereby preparing an isolated, purified Y4 receptor.

[0025] This invention also provides a method of preparing the isolated human or rat Y4 receptor which comprises inserting the nucleic acid encoding a human or rat Y4 receptor as mentioned above in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the receptor produced by the resulting cell, and purifying the receptor so recovered.

[0026] This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes the Y4 receptor as mentioned above so as to prevent translation of mRNA molecules which encode the Y4 receptor.

Figure 1

Nucleotide Sequence and Deduced Amino Acid Sequence of a Novel Human hp25a Neuropeptide Receptor (Sequence I.D. Nos. 1 and 2). Nucleotides are presented in the 5' to 3' orientation and the coding region is numbered starting from the initiating methionine and ending in the termination codon. Deduced amino acid sequence by translation of a long open reading frame is shown, along with the 5' and 3' untranslated regions. Numbers in the left and right margins represent nucleotide (top line) and amino acid (bottom line) numberings, starting with the first position as the adenosine (A) and the initiating methionine (M), respectively.

Figure 2

Sequence Alignment of the Human hp25a clone with human Y1, rat Y1, and mouse Y1 receptor genes. The deduced amino acid sequence of the human hp25a (Y4) receptor (first line), from the starting methionine (M) to the stop codon (*), is aligned with the human Y1 receptor clone (Larhammar at al., 1992), rat Y1 receptor clone (Eva et al., 1990), and mouse Y1 receptor clone (Eva et al., 1992). Hyphens represent added spaces necessary for proper alignment. Gray shading indicates residues in receptor clones which are identical to hp25a. Numbers above amino acid sequences correspond to amino acid positions of hp78a, starting with the initiating methionine (M) and ending with the termination codon (*), and including spaces to account for proper alignment. Solid bars above the sequence indicate the seven putative transmembrane (TM) spanning regions (TM I - VII).

Figure 3. Nucleotide sequence and deduced amino acid sequence of the rat Y4 receptor encoded by rs16b (Sequence I.D. Nos and). Nucleotides are presented in the 5' to 3' orientation and the coding region is numbered starting from the putative initiating methionine and ending in the termination codon. Deduced amino acid sequence by translation of a long open reading frame is shown, along with 5' and 3' untranslated regions. The amino acid sequence is represented using single-letter abbreviations.

Figure 4. Alignment of rat and human Y4 receptors. Predicted amino acid sequences of the rat Y4 receptor (Y4rat) and human Y4 receptor (Y4hum) are shown; the sequences are 75% identical overall and 84% identical in the transmembrane domains. Single letter abbreviations for amino acids are shown. The seven putative transmembrane (TM) spanning regions (TM I - VII) are indicated by brackets above the sequence.

Figure 5

Equilibrium binding of ^{125}I-PYY to membranes from COS-7 cells transiently expressing hp25a receptors. Membranes were incubated with ^{125}I-PYY for the times indicated, in the presence or absence of 100 nM human PP. Specific binding, B, was plotted against time, t, to obtain the maximum number of equilibrium binding sites, B_t, and observed association rate, K_{obs}, according to the equation, $B = B_t * (1 - e^{-(k_{obs} t)})$. Binding is shown as the percentage of total equilibrium binding, B_t, determined by nonlinear regression analysis. Data are representative of three independent experiments, with each point measured in triplicate.

Figure 6A

Saturable equilibrium binding of ^{125}I-PYY to membranes from COS-7 cells transiently expressing hp25a receptors. Membranes were incubated with ^{125}I-PYY ranging in concentration from 0.003 nM to 2 nM, in the presence or absence of 100 nM human PP.

Figure 6B

Specific binding of the ^{125}I-PYY to membranes from COS-7 cells transiently expressing hp25a receptors under the conditions described in Figure 6A was plotted against the free ^{125}I-PYY concentration, [L], to obtain the maximum number of saturable binding sites,B_{max}, and the ^{125}I-PYY equilibrium dissociation constant, K_d, according to the binding isotherm, $B = B_{max}[L]/([L] + K_d)$. Specific binding is shown for data from a representative of four independent experiments, with each point measured in quadruplicate.

Figure 7. Competitive displacement of ^{125}I-PYY from COS-7 cells transiently expressing hp25a receptors. Membranes were incubated with ^{125}I-PYY and increasing concentrations of peptide competitors. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to K_i values according to the equation, $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the ^{125}I-PYY concentration and K_d is the equilibrium dissociation constant of ^{125}I-PYY. Data are representative of at least two independent experiments, with each point measured once or in duplicate. Rank orders of affinity for these and other compounds are listed separately in Table 2.

Figure 8. Inhibition of forskolin-stimulated cAMP accumulation in intact LM(tk-) cells stably expressing the human Y4 receptor. Functional data were derived from radioimmunoassay of cAMP in LM(tk-) cells stimulated with 10 µM forskolin over a 5 minute period. Human PP was tested for agonist activity at concentrations ranging from 0.03 pM to 0.3 µM over the same period. Data were fit to a four parameter logistic equation by nonlinear regression. The data shown are representative of three independent experiments.

Figures 9A and 9B. Figure 9A. Stimulation of intracellular free calcium concentration in intact LM(tk-) cells stably expressing the human Y4 receptor. Representative time course. Functional data were derived from Fura-2/AM fluorescence in LM(tk-) cells stimulated with 100 nM human PP (open squares) or 100 nM human NPY (closed squares) at the time indicated by the arrow. The data shown are representative of two independent experiments. Figure 9B. Concentration/response curve. Data were fit to a four parameter logistic equation by nonlinear regression."

[0027] Throughout this application, the following standard abbreviations are used to indicate specific nucleotide bases:

C = cytosine A = adenine
T = thymine G = guanine

[0028] This invention provides isolated nucleic acid molecules which encode human or rat Y4 receptors. The human receptor is a receptor characterized by a pharmacological profile characteristic of the human Y4 receptor as shown in Table 6. The rat receptor is for example a receptor characterized by a pharmacological profile characteristic of the rat Y4 receptor as shown in Table 6. The isolated nucleic acid molecule may encode a Y4 receptor being characterized by an amino acid sequence in the transmembrane region, wherein the amino acid sequence has 60% homology or higher to the amino acid sequence in the transmembrane region of the human Y4 receptor shown in Figure 2. In one embodiment, the Y4 receptor has substantially the same amino acid sequence as the human Y4 receptor as described in Figure 1. In yet another embodiment, the Y4 receptor has substantially the same amino acid sequence as the rat Y4 receptor as described in Figure 3. In another embodiment, the Y4 receptor has the amino acid sequence as shown in Figure 1. In another embodiment, the Y4 receptor has the amino acid sequence as shown in Figure 3. As used herein, the term Y4 receptor encompasses any amino acid sequence, polypeptide or protein having substantially the same pharmacology provided by subject human Y4 receptor as shown in Tables 1-3 and Table 6 and Figures 5-7. As

described herein the human Y4 receptor has a pharmacological profile that differs from any known neuropeptide Y receptor subtype (i.e. Y1, Y2 and Y3), Neuropeptide YY receptor, and pancreatic polypeptide receptor, and is therefore designated as the human Y4 receptor.

[0029] The only NPY receptor which has been cloned to date is the Y1 receptor gene, from mouse (Eva et al., 1992), rat (Eva et al., 1990), and human (Larhammar et al., 1992). The Y4 receptor's greatest homology with any known receptor disclosed in the Genbank/EMBL databases is a 42% overall amino acid identity with the human Y1 receptor.

[0030] This invention provides an isolated nucleic acid molecule encoding a human Y4 receptor or a rat Y4 receptor having the characteristics as mentioned above. As used herein, the term "isolated nucleic acid molecule" means a nucleic acid molecule that is a molecule in a form which does not occur in nature. Examples of such an isolated nucleic acid molecule are an RNA, cDNA, or isolated genomic DNA molecule encoding a Y4 receptor. One means of isolating a human Y4 receptor is to probe a human genomic library with a natural or artificially designed DNA probe, using methods well known in the art. DNA probes derived from the human receptor gene Y4 are particularly useful probes for this purpose. DNA and cDNA molecules which encode human Y4 receptors may be used to obtain complementary genomic DNA, cDNA or RNA from human, mammalian or other animal sources, or to isolate related cDNA or genomic clones by the screening of cDNA or genomic libraries, by methods described in more detail below. Transcriptional regulatory elements from the 5' untranslated region of the isolated clones, and other stability, processing, transcription, translation, and tissue specificity-determining regions from the 3' and 5' untranslated regions of the isolated genes are thereby obtained. Examples of a nucleic acid molecule are an DNA, RNA, cDNA, or isolated genomic DNA molecule or synthetic DNA or RNA molecules encoding a human or rat Y4 receptor. Such molecules may have coding sequences such as the coding sequences shown in Figures 1 or 3. The DNA molecule of Figure 1 encodes the amino acid sequence of a human Y4 receptor protein, while the DNA molecule of Figure 3 encodes one amino acid sequence of the rat Y4 receptor.

[0031] This invention further provides a cDNA molecule encoding a Y4 receptor having a coding sequence substantially the same as the coding sequence shown in Figures 1 and 3. This molecule is obtained by the means described above.

[0032] This invention also provides an isolated protein which is a human Y4 receptor or a rat Y4 receptor encoded by the nucleic acid molecule as mentioned above. As used herein, the term "isolated protein" means a protein molecule free of other cellular components. An example of such a protein is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1 which is a human Y4 receptor or the amino acid sequence shown in Figure 3 which is a rat Y4 receptor. One means for obtaining isolated Y4 receptor is to express DNA encoding the receptor as mentioned above in a suitable host, such as a bacterial, yeast, insect or mammalian cell, using methods well known in the art, and recovering the receptor protein after it has been expressed in such a host, again using methods well known in the art. The receptor may also be isolated from cells which express it, in particular from cells which have been transfected with the expression vectors described below in more detail.

[0033] This invention provides vectors comprising isolated nucleic acid molecules such as DNA, RNA, or cDNA encoding a human Y4 receptor or rat Y4 receptor as mentioned above. Examples of vectors are viruses such as bacteriophages (such as phage lambda), animal viruses (such as Herpes virus, Murine Leukemia virus, and Baculovirus), cosmids, plasmids (such as pUC18, available from Pharmacia, Piscataway, NJ), and other recombination vectors. Nucleic acid molecules are inserted into vector genomes by methods well known in the art. For example, insert and vector DNA can both be exposed to a restriction enzyme to create complimentary ends on both molecules which base pair with each other and are then ligated together with a ligase. Alternatively, linkers can be ligated to the insert DNA which correspond to a restriction site in the vector DNA, which is then digested with the restriction enzyme which cuts at that site. Other means are also available. Specific examples of such plasmids are plasmids comprising cDNA having a coding sequence substantially the same as the coding sequence shown in Figure 1 and designated clone hp25a (Seq. I.D. No. 1) or the coding sequence shown in Figure 3 and designated clone rs16b (Sequence I.D. No. 27).

[0034] This invention also provides vectors comprising DNA molecules encoding Y4 receptors as mentioned above, adapted for expression in a bacterial cell, a yeast cell, an insect cell or a mammalian cell which additionally comprise the regulatory elements necessary for expression of the DNA in the bacterial, yeast, insect or mammalian cells operatively linked to the DNA encoding a Y4 receptor as to permit expression thereof. DNA having coding sequences substantially the same as the coding sequence shown in Figure 1 may usefully be inserted into the vectors to express human Y4 receptors. DNA having coding sequences substantially the same as the coding sequence shown in Figure 3 may usefully be inserted into the vectors to express rat Y4 receptors. Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG (Maniatis, et al., Molecular Cloning, Cold Spring Harbor Laboratory, 1982).

[0035] Similarly, a eukaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribos-

ome. Furthermore, an insect expression vector, such as recombinant Baculovirus, uses the polyhedrin gene expression signals for expression of the inserted gene in insect cells. Such vectors may be obtained commercially or assembled from the sequences described by methods well known in the art, for example the methods described above for constructing vectors in general. Expression vectors are useful to produce cells that express the receptor. Certain uses for such cells are described in more detail below.

[0036] This invention further provides a plasmid adapted for expression in a bacterial, yeast, insect, or, in particular, a mammalian cell which comprises a DNA molecule encoding a Y4 receptor as mentioned above and the regulatory elements necessary for expression of the DNA in the bacterial, yeast, insect, or mammalian cell operatively linked to the DNA encoding a Y4 receptor as to permit expression thereof. Some plasmids adapted for expression in a mammalian cell are pSVL (available from Pharmacia, Piscataway, NJ) and pcEXV-3 (Miller J. and Germain R.N., J. Exp. Med. 164:1478 (1986)). A specific example of such plasmid is a plasmid adapted for expression in a mammalian cell comprising cDNA having coding sequences substantially the same as the coding sequence shown in Figure 1 and the regulatory elements necessary for expression of the DNA in the mammalian cell which is designated pcEXV-Y4 and deposited under ATCC Accession No. 75631. Another example of such plasmid is a plasmid adapted for expression in a mammalian cell comprising cDNA having coding sequences substantially the same as the coding sequence shown in Figure 3 and the regulatory elements necessary for expression of the DNA in the mammalian cell which is designated pcEXV-rY4 and deposited with the ATCC Those skilled in the art will readily appreciate that numerous plasmids adapted for expression in a mammalian cell which comprise the DNA encoding Y4 receptors as mentioned above and the regulatory elements necessary to express such DNA in the mammalian cell may be constructed utilizing existing plasmids and adapted as appropriate to contain the regulatory elements necessary to express the DNA in the mammalian cell. The plasmids may be constructed by the methods described above for expression vectors and vectors in general, and by other methods well known in the art.

[0037] The deposit discussed supra, and the other deposits discussed herein, were made pursuant to, and in satisfaction of, the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockyille, Maryland 20852.

[0038] This invention provides a cell comprising a nucleic acid encoding a Y4 receptor as mentioned above, such as a mammalian cell comprising a plasmid adapted for expression in a mammalian cell, which comprises a nucleic acid molecule encoding a Y4 receptor as mentioned above, the protein encoded thereby is expressed on the cell surface, and the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding a Y4 receptor as to permit expression thereof. Numerous mammalian cells may be used as hosts, including, for example, the mouse fibroblast cell NIH-3T3, CHO cells, HeLa cells, LM(tk-) cells, Y1 cells, etc. Expression plasmids such as that described supra may be used to transfect mammalian cells by methods well known in the art such as calcium phosphate precipitation, or DNA encoding these Y4 receptors may be otherwise introduced into mammalian cells, e.g., by microinjection, to obtain mammalian cells which comprise DNA, e.g., cDNA or a plasmid, encoding either Y4 receptor. An example for a LM(tk-) cell is designated L-hY4-3 (deposited with ATCC). An another example for an NIH-3t3 cell is designated N-hY4-5 (deposited with ATCC).

[0039] For convenience it is established that in the following the term "Y4 receptor" refers to a human or rat Y4 receptor characterized as stated in claims 1 and 2 and the term "nucleic acid encoding an Y4 receptor" refers to a nucleic acid encoding a human or rat Y4 receptor characterized by the features as outlined in claims 1 to 9.

[0040] This invention provides a method for determining whether a ligand can specifically bind to a Y4 receptor which comprises contacting a plurality of cells transfected with and expressing nucleic acid encoding the Y4 receptor with the ligand under conditions permitting binding of ligands to such receptor, and detecting the presence of any such ligand bound specifically to the Y4 receptor, thereby determining whether the ligand binds specifically to a Y4 receptor.

[0041] This invention provides a method for determining whether a ligand can bind specifically to a Y4 receptor which comprises preparing a cell extract from cells transferred with and expressing nucleic acid encoding a Y4 receptor, isolating a membrane fraction from the cell extract, contacting the ligand with the membrane fraction under conditions permitting binding of ligands to such conditions permitting binding of ligands to such receptor, and detecting the presence of any ligand bound to the Y4 receptor, thereby determining whether the compound is capable of specifically binding to a Y4 receptor.

[0042] This invention provides a method for determining whether a ligand is a Y4 receptor agonist which comprises contacting a plurality of cells transfected with and expressing nucleic acid encoding a Y4 receptor with the ligand under conditions permitting the activation of a functional Y4 receptor response from the cell, and detecting by means of a bioassay, such as a second messenger response, an increase in Y4 receptor activity, thereby determining whether the ligand is a Y4 receptor agonist.

[0043] This invention provides a method for determining whether a ligand is a Y4 receptor agonist which comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding a Y4 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand under conditions permitting the

activation of a functional Y4 receptor response, and detecting by means of a bioassay, such as a second messenger response, an increase in Y4 receptor activity, thereby dtermining whether the ligand is a Y4 receptor agonist.

[0044] This invention provides a method for determining whether a ligand is a Y4 receptor antagonist which comprises contacting a plurality of cells transfected with and expressing nucleic acid encoding a Y4 receptor with the ligand in the presence of a known Y4 receptor agonist, such as PP, under conditions permitting the activation of a functional Y4 receptor response and detecting by means of a bioassay, such as a second messenger response, a decrease in Y4 receptor activity, thereby determining whether the ligand is a Y4 receptor antagonist.

[0045] This invention provides a method for determining whether a ligand is a Y4 receptor antagonist which comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding a Y4 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand in the presence of a known Y4 receptor agonist, such as PP, under conditions permitting the activation of a functional Y4 receptor response and detecting by means of a bioassay, such as a second messenger response, a decrease in Y4 receptor activity, thereby determining whether the ligand is a Y4 receptor antagonist.

[0046] As used herein, the term "agonist" means any ligand capable of increasing Y4 receptor activity. As used herein, the term "antagonist" means any ligand capable of decreasing Y4 receptor activity. This compound may be natural or synthetic.

[0047] In one embodiment of the above-described methods, the cell is a mammalian cell. In a further embodiment, the cell is non-neuronal in origin. In another embodiment, the nonneuronal cell is a COS-7 cell, a CHO cell, an NIH-3T3 cell or an LM(tk-) cell.

[0048] One method for determining whether a ligand is capable of binding to the human Y4 receptor comprises contacting a transfected nonneuronal cell (i.e. a cell that does not naturally express any type of NPY, PP, or PYY receptor, thus will only express such a receptor if it is transfected into the cell) expressing a Y4 receptor on its surface, or contacting a membrane preparation derived from such a transfected cell, with the ligand under conditions which are known to prevail, and thus to be associated with in vivo binding of the ligands to a Y4 receptor, detecting the presence of any of the ligand being tested bound to the Y4 receptor on the surface of the cell, and thereby determining whether the ligand binds to, activates or inhibits the activation of the Y4 receptor. A response system for detecting the activation or inhibition of activation of the Y4 receptor is obtained by transfection of isolated DNA into a suitable host cell containing the desired second messenger system such as phosphoinositide hydrolysis, adenylate cyclase, guanylate cyclase or ion channels. Such a suitable host cell system is isolated from pre-existing cell lines, or can be generated by inserting appropriate components of second messenger systems into existing cell lines. Such a transfection system provides a complete response system for investigation or assay of the activity of Y4 receptors with ligands as described above. Transfection systems are useful as living cell cultures for competitive binding assays between known or candidate drugs and ligands which bind to the receptor and which are labeled by radioactive, spectroscopic or other reagents. Membrane preparations containing the receptor isolated from transfected cells are also useful for these competitive binding assays. Functional assays of second messenger systems or their sequelae in transfection systems act as assays for binding affinity and efficacy in the activation of receptor function. A transfection system constitutes a "drug discovery system" useful for the identification of natural or synthetic compounds with potential for drug development that can be further modified or used directly as therapeutic compounds to activate or inhibit the natural functions of the Y4 receptor. The transfection system is also useful for determining the affinity and efficacy of known drugs at the Y4 receptor sites.

[0049] This invention also provides a method of screening chemical compounds to identify drug candidates which specifically bind to a Y4 receptor on the surface of a cell which comprises contacting a plurality of cells transfected with and expressing nucleic acid encoding a Y4 receptor with a plurality of compounds, and determining those compounds which bind to the cell, thereby identifying drugs which specifically bind to a Y4 receptor.

[0050] This invention also provides a method of screening chemical compounds to identify drug candidates which specifically bind to a Y4 receptor on the surface of a cell which comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding a Y4 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of compounds and determining those compounds which bind to the membrane fraction, thereby identifying drug candidates which specifically bind to a Y4 receptor.

[0051] This invention also provides a method of screening chemical compounds to identify drug candidates which act as Y4 receptor agonists which comprises contacting a plurality of cells transfected with and expressing nucleic acid encoding a Y4 receptor with a plurality of compounds under conditions permitting the activation of a functional Y4 receptor response, determining those compounds which activate the Y4 receptor in the cell using a bioassay, such as a second messenger assay, thereby identifying drugs which act as Y4 receptor agonists.

[0052] This invention also provides a method of screening chemical compounds to identify drug candidates which act as Y4 receptor agonists which comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding a Y4 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of compounds under conditions permitting the activation of a functional Y4 receptor response, determining those compounds which activate the Y4 receptor in the cell using a bioassay, such as a second messenger assay,

thereby identifying drug candidates which act as Y4 receptor agonists.

[0053] This invention also provides a method of screening chemical compounds to identify drug candidates which act as Y4 receptor antagonists which comprises contacting a plurality of cells transfected with and expressing DNA encoding a Y4 receptor with a plurality of compounds in the presence of a known Y4 receptor agonist, such as PP, under conditions permitting the activation of a functional Y4 receptor response, and determining those compounds which inhibit the activation of the Y4 receptor in the cell using a bioassay, such as a second messenger assay, thereby identifying drug candidates which act as Y4 receptor antagonists. This invention also provides a method of screening chemical compounds to identify drug candidates which act as Y4 receptor antagonists which comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding a Y4 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of compounds in the presence of a known Y4 receptor agonist, such as PP, under conditions permitting the activation of a functional Y4 receptor response, and determine those compounds which inhibit the activation of the Y4 receptor in the cell using a bioassay, such as a second messenger assay, thereby identifying drug candidates which act as Y4 receptor antagonists.

[0054] In one embodiment of the above identified methods, the cell is a mammalian cell. In another embodiment, the mammalian cell is non-neuronal in origin. In a further embodiment, the mammalian cell non-neuronal in origin is a Cos-7 cell, a CHO cell, an LM(tk-) cell, a Y1 murine adrenal cell, or an NIH-3T3 cell.

[0055] The nucleic acid in the cell may have a coding sequence substantially the same as the coding sequences shown in Figures 1 and 3. Drug candidates are identified by choosing chemical compounds which bind with high affinity to the expressed Y4 receptor protein in transfected cells, using radioligand binding methods well known in the art, examples of which are shown in the binding assays described herein. Drug candidates are also screened for selectivity by identifying compounds which bind with high affinity to the Y4 receptor but do not bind with high affinity to any other NPY receptor subtype or to any other known receptor site. Because selective, high affinity compounds interact primarily with the target Y4 receptor site after administration to the patient, the chances of producing a drug with unwanted side effects are minimized by this approach.

[0056] This invention provides a pharmaceutical composition as mentioned below comprising a chemical compound and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. Once the candidate drug has been shown to be adequately bio-available following a particular route of administration, for example orally or by injection (adequate therapeutic concentrations must be maintained at the site of action for an adequate period to gain the desired therapeutic benefit), and has been shown to be non-toxic and therapeutically effective in appropriate disease models, the drug may be administered to patients by that route of administration determined to make the drug bio-available, in an appropriate solid or solution formulation, to gain the desired therapeutic benefit.

[0057] This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the coding sequence of a nucleic acid molecule encoding a Y4 receptor, for example with a coding sequence included within the sequences shown in Figures 1 and 3. As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. As used herein, a "unique sequence" is a sequence specific to only the nucleic acid molecules encoding a Y4 receptor. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. Detection of nucleic acid encoding human Y4 receptors is useful as a diagnostic test for any disease process in which levels of expression of the corresponding Y4 receptor is altered. Nucleic acid probe molecules are produced by insertion of a nucleic acid molecule which encodes a Y4 receptor or fragments thereof into suitable vectors, such as plasmids or bacteriophages, followed by insertion into suitable bacterial host cells and replication and harvesting of the nucleic acid probes, all using methods well known in the art. For example, the nucleic acid may be extracted from a cell lysate using phenol and ethanol, digested with restriction enzymes corresponding to the insertion sites of the nucleic acid into the vector (discussed above), electrophoresed, and cut out of the resulting gel. Example of such nucleic acid molecules are shown in Figures 1 and 3. The probes are useful for 'in situ' hybridization or in order to locate tissues which express this gene family, or for other hybridization assays for the presence of these genes or their mRNA in various biological tissues. In addition, synthesized oligonucleotides (produced by a DNA synthesizer) complementary to the sequence of a DNA molecule which encodes a Y4 receptor of are useful as probes for these genes, for their associated mRNA, or for the isolation of related genes by homology screening of genomic or cDNA libraries, or by the use of amplification techniques such as the Polymerase Chain Reaction. Synthesized oligonucleotides as described may also be used to determine the cellular localization of the mRNA produced by the Y4 gene by in situ hybridization.

[0058] This invention also provides a method of detecting expression of a Y4 receptor by detecting the presence of mRNA coding for a Y4 receptor which comprises obtaining total mRNA from the cell using methods well known in the

art and contacting the mRNA so obtained with a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a Y4 receptor under hybridizing conditions, and detecting the presence of mRNA hybridized to the probe, thereby detecting the expression of the Y4 receptor by the cell. Hybridization of probes to target nucleic acid molecules such as mRNA molecules employs techniques well known in the art. In one possible means of performing this method, nucleic acids are extracted by precipitation from lysed cells and the mRNA is isolated from the extract using a column which binds the poly-A tails of the mRNA molecules. The mRNA is then exposed to radioactively labelled probe on a nitrocellulose membrane, and the probe hybridizes to and thereby labels complementary mRNA sequences. Binding may be detected by autoradiography or scintillation counting. However, other methods for performing these steps are well known to those skilled in the art, and the discussion above is merely an example.

[0059] This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing with any sequences of an mRNA molecule which encodes a Y4 receptor so as to prevent translation of the mRNA molecule. The antisense oligonucleotide may have a sequence capable of specifically hybridizing with any sequences of the cDNA molecule whose sequence is shown in Figure 1 or Figure 3. A particular example of an antisense oligonucleotide is an antisense oligonucleotide comprising chemical analogues of nucleotides.

[0060] This invention also provides a pharmaceutical composition comprising an amount of the oligonucleotide described above effective to reduce activity of a human Y4 receptor by passing through a cell membrane and specifically binding with mRNA encoding a Y4 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable carrier capable of passing through a cell membrane. The oligonucleotide may be coupled to a substance which inactivates mRNA, such as a ribozyme. The pharmaceutically acceptable carrier capable of passing through cell membranes may also comprise a structure which binds to a receptor on a cell capable of being taken up by cells after binding to the structure. The structure or the pharmaceutically acceptable carrier may be capable of binding to a receptor which is specific for a selected cell type. The structure may be part of a protein known to bind a cell-type specific receptor, for example an insulin molecule, which would target pancreatic cells. Nucleic molecules having coding sequences substantially the same as the coding sequences shown in Figures 1 and 3 may be used as the oligonucleotides of the pharmaceutical composition.

[0061] This invention also provides the use of a chemical compound for the preparation of a pharmaceutical composition for treating an abnormality wherein the abnormality is alleviated by decreasing the activity of a human Y4 receptor which treating comprises administering to a subject an amount of the pharmaceutical composition described above effective to decrease the activity of the Y4 receptor. Several examples of such abnormal conditions are amnesia, anxiety, epilepsy, pain, hypertension, locomotor problems, circadian rhythm disorders, eating/body weight disorders, sexual/reproductive disorders, nasal congestion, diarrhea, gastrointestinal and cardiovascular disorders, and sleep and eating disorders.

[0062] Antisense oligonucleotide drugs inhibit translation of mRNA encoding these receptors. Synthetic oligonucleotides, or other antisense chemical structures are designed to bind to mRNA encoding the Y4 receptor and inhibit translation of mRNA and are useful as drugs to inhibit expression of Y4 receptor genes in patients. This invention provides a means to therapeutically alter levels of expression of human Y4 receptors by the use of a synthetic antisense oligonucleotide drug (SAOD) which inhibits translation of mRNA encoding these receptors. Synthetic oligonucleotides, or other antisense chemical structures designed to recognize and selectively bind to mRNA, are constructed to be complementary to portions of the nucleotide sequences shown in Figures 1 and 3 of DNA, RNA or of chemically modified, artificial nucleic acids. The SAOD is designed to be stable in the blood stream for administration to patients by injection, or in laboratory cell culture conditions, for administration to cells removed from the patient. The SAOD is designed to be capable of passing through cell membranes in order to enter the cytoplasm of the cell by virtue of physical and chemical properties of the SAOD which render it capable of passing through cell membranes (e.g. by designing small, hydrophobic SAOD chemical structures) or by virtue of specific transport systems in the cell which recognize and transport the SAOD into the cell. In addition, the SAOD can be designed for administration only to certain selected cell populations by targeting the SAOD to be recognized by specific cellular uptake mechanisms which binds and takes up the SAOD only within certain selected cell populations. For example, the SAOD may be designed to bind to a receptor found only in a certain cell type, as discussed above. The SAOD is also designed to recognize and selectively bind to the target mRNA sequence, which may correspond to a sequence contained within the sequences shown in Figures 1 and 3 by virtue of complementary base pairing to the mRNA. Finally, the SAOD is designed to inactivate the target mRNA sequence by any of three mechanisms: 1) by binding to the target mRNA and thus inducing degradation of the mRNA by intrinsic cellular mechanisms such as RNAse I digestion, 2) by inhibiting translation of the mRNA target by interfering with the binding of translation-regulating factors or of ribosomes, or 3) by inclusion of other chemical structures, such as ribozyme sequences or reactive chemical groups, which either degrade or chemically modify the target mRNA. Synthetic antisense oligonucleotide drugs have been shown to be capable of the properties described above when directed against mRNA targets (J.S. Cohen, Trends in Pharm. Sci. 10, 435 (1989); H.M. Weintraub, Sci. Am. January (1990) p. 40). In addition, coupling of ribozymes to antisense oligonucleotides is a promising strategy for inactivating target

mRNA (N. Sarver et al., Science 247, 1222 (1990)). An SAOD serves as an effective therapeutic agent if it is designed to be administered to a patient by injection, or if the patient's target cells are removed, treated with the SAOD in the laboratory, and replaced in the patient. In this manner, an SAOD serves as a therapy to reduce receptor expression in particular target cells of a patient, in any clinical condition which may benefit from reduced expression of Y4 receptors.

[0063] This invention provides an antibody directed to a Y4 receptor, for example a monoclonal antibody directed to an epitope of a Y4 receptor present on the surface of a cell and having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human Y4 receptor included in the amino acid sequence shown in Figure 1 (Seq. I.D. No. 2) or the rat Y4 receptor included in the amino acid sequence shown in Figure 3 (Seq.I.D. No. 28). Amino acid sequences may be analyzed by methods well known in the art to determine whether they produce hydrophobic or hydrophilic regions in the proteins which they build. In the case of cell membrane proteins, hydrophobic regions are well known to form the part of the protein that is inserted into the lipid bilayer which forms the cell membrane, while hydrophilic regions are located on the cell surface, in an aqueous environment. Therefore antibodies to the hydrophilic amino acid sequences shown in Figures 1 and 3 will probably bind to a surface epitope of a human or rat Y4 receptor, respectivley, as described. Antibodies directed to Y4 receptors may be serum-derived or monoclonal and are prepared using methods well known in the art. For example, monoclonal antibodies are prepared using hybridoma technology by fusing antibody producing B cells from immunized animals with myeloma cells and selecting the resulting hybridoma cell line producing the desired antibody. Cells such as COS-7 cells or LM(tk-) cells comprising DNA encoding the human Y4 receptor and thereby expressing the human Y4 receptor may be used as immunogens to raise such an antibody. Alternatively, synthetic peptides may be prepared using commercially available machines and the amino acid sequences shown in Figures 1 and 3 (Seq. I.D. Nos. 2 and 28). As a still further alternative, DNA, such as a cDNA or a fragment thereof, may be cloned and expressed and the resulting polypeptide recovered and used as an immunogen. These antibodies are useful to detect the presence of human Y4 receptors encoded by the isolated DNA, or to inhibit the function of the receptors in living animals, in humans, or in biological tissues or fluids isolated from animals or humans.

[0064] This invention provides a pharmaceutical composition which comprises an amount of an antibody directed to the human Y4 receptor effective to block binding of ligands to the Y4 receptor, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to an epitope of a Y4 receptor present on the surface of a cell and having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the Y4 receptor included in the amino acid sequences shown in Figures 1 and 3 is useful for this purpose.

[0065] This invention also provides the use of a chemical compound for the preparation of a pharmaceutical composition for treating an abnormality wherein the abnormality is alleviated by decreasing the activity of a Y4 receptor which treating comprises administering to a subject an amount of the pharmaceutical composition described above effective to block binding of ligands to the Y4 receptor, thereby treating the abnormality. Binding of the antibody to the receptor prevents the receptor from functioning, thereby neutralizing the effects of Y4 receptor activity. The monoclonal antibodies described above are both useful for this purpose..Some examples of abnormalities are amnesia, depression, anxiety, epilepsy, pain, depression, hypertension, and sleep and eating disorders.

[0066] This invention provides a method of detecting the presence of a Y4 receptor on the surface of a cell which comprises contacting the cell with an antibody directed to the Y4 receptor, under conditions permitting binding of the antibody to the receptor, and detecting the presence of the antibody bound to the cell, thereby detecting the presence or the Y4 receptor on the surface of the cell. Such a method is useful for determining whether a given cell is defective in activity of Y4 receptors on the surface of the cell. Bound antibodies are detected by methods well known in the art, for example by binding fluorescent markers to the antibodies and examining the cell sample under a fluorescence microscope to detect fluorescence on a cell indicative of antibody binding. The monoclonal antibodies described above are useful for this purpose.

[0067] This invention further provides the use of a chemical compound for the preparation of a pharmaceutical composition for treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a Y4 receptor which treating comprises administering to the subject an effective amount of the pharmaceutical composition thereby treating the abnormality.

[0068] This invention further provides the use of a chemical compound for the preparation of a pharmaceutical composition for treating an abnormality in a subject wherein the abnormality is alleviated by activation of a Y4 receptor which comprises administering to a subject an effective amount of the pharmaceutical composition, thereby treating the abnormaility.

[0069] This invention provides a composition comprising the nucleic acid probe as mentioned above for diagnosing a predisposition to a disorder associated with the activity of a specific Y4 receptor allele which comprises: a) obtaining DNA of subjects suffering from the disorder; b) performing a restriction digest of the DNA with a panel of restriction enzymes; c.electrophoretically separating the resulting DNA fragments on a sizing gel; d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a Y4 receptor and labelled with a detectable marker; e) detecting labelled bands which have hybridized to the DNA encoding a Y4 receptor labelled with a

detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f) preparing DNA obtained for diagnosis by steps a-e; and g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and thereby to diagnose predisposition to the disorder if the patterns are the same. This method may also be used to diagnose a disorder associated with the expression of a specific Y4 receptor allele.

[0070] This invention provides a method of preparing the purified, isolated Y4 receptor which comprises a) constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of nucleic acid in the cell operatively linked to the nucleic acid encoding a Y4 receptor as to permit expression thereof, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, insect cells and mammalian cells; b) inserting the vector of step a in a suitable host cell; c) incubating the cells of step b under conditions allowing the expression of a Y4 receptor; d) recovering the receptor so produced; and e) purifying the receptor so recovered, thereby preparing the purified, isolated Y4 receptor. An example of an isolated Y4 receptor is an isolated protein having substantially the same amino acid sequence as the amino acid sequences shown in Figures 1 and 3. For example, cells can be induced to express receptors by exposure to substances such as hormones. The cells can then be homogenized and the receptor isolated from the homogenate using an affinity column comprising, for example, PP or another substance which is known to bind to the receptor. The resulting fractions can then be purified by contacting them with an ion exchange column, and determining which fraction contains receptor activity or binds anti-receptor antibodies. This method for preparing Y4 receptor uses recombinant DNA technology methods well known in the art. For example, isolated nucleic acid encoding Y4 receptor is inserted in a suitable vector, such as an expression vector. A suitable host cell, such as a bacterial cell, or a eukaryotic cell such as a yeast cell, is transfected with the vector. Y4 receptor is isolated from the culture medium by affinity purification or by chromatography or by other methods well known in the art.

[0071] This invention identifies for the first time a new receptor protein, its amino acid sequence, and its human gene. Furthermore, this invention describes a previously unrecognized group of receptors within the definition of a Y4 receptor. The information and experimental tools provided by this discovery are useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for this new receptor protein, its associated mRNA molecule or its associated genomic DNA. The information and experimental tools provided by this discovery will be useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for this new receptor protein, its associated mRNA molecule, or its associated genomic DNA.

[0072] Specifically, this invention relates to the first isolation of a human and a rat genomic clone encoding a Y4 receptor. A new human gene for the receptor identified herein as Y4 has been identified and characterized. In addition, the human Y4 receptor has been expressed in COS-7 cells. The pharmacological binding properties of the protein encoded have been determined, and these binding properties classify this protein as a novel NPY/PYY/PP receptor which we designate as a Y4 receptor. Mammalian cell lines expressing this Y4 receptor at the cell surface have been constructed, thus establishing the first well-defined, cultured cell lines with which to study this Y4 receptor.

[0073] This invention will be better understood by reference to the Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

[0074] Cloning and Sequencing of a human (Y4) Neuropeptide Receptor. A human placenta genomic library in λ dash II (≈1.5 x 10^6 total recombinants; Stratagene, LaJolla, CA) was screened using overlapping transmembrane (TM) oligonucleotide probes (TM 1, 2, 3, 5 and 7) derived from the rat Y1 neuropeptide receptor gene (Eva, C. et al., 1990; GenBank accession No. Z11504). Overlapping oligomers (TM1: nts. 198-251, (+)strand/5'-TTGCTTATGGGGCTGTGATTATTCTTGGGGTCTCTGGAAACCTGG-3' (Sequence I.D. No. 3) and (-)strand/5'-TAGGATGATTATGATCAATGCCAGGTTTCCAGAGACCCCAAGAAT-3' (Sequence I.D. No. 4); TM2: nts. 269-328, (+)strand/5'-AAAGAGATGAGGAATGTCACCAACATTCTGATCGT
GAACCTCTCC-3' (Sequence I.D. No. 5) and (-)strand/5'-CAGCAAGTCTGAGAAGGAGAGGTTCAC-GATCAGAATGTTGGTGAC-3' (Sequence I.D. No. 6); TM3: nts. 401-478, (+)strand/5'-TGCAAACTGAATCCTTTTGTGCAATGCGTCTCCATTACAGTATCCATTTTCTCT-3' (Sequence I.D. No. 7) and (-)strand/5'-ACGTTCCACAGC GATGAGAACCAGAGAGAAAATGGATACTGTAATGGAGACGCA-3' (Sequence I.D. No. 8); TM5: nts. 716-778, (+)strand/5'-CTGCAGTATTTTGGCCCACTCTGTTTCATATTCATATGCTAC-3' (Sequence I.D. No. 9) and (-)strand/5'-CAAGCGAATGTATATCTTGAAGTAGCATATGAATATGAAACA-3' (Sequence I.D. No. 10); TM7: nts. 971-1045, (+)strand/5'-CTGCTCTGCCACCTCACGGCCATGATCTCCACCTGCGTCAACC
CCATC-3' (Sequence I.D. No. 11) and (-)strand/5'-GAAATTTTTGTTCAGGAATCCATAAAAGATGGGGTTGA
CGCAGGTGGA-3' (Sequence I.D. No. 12); GenBank accession No. Z11504) were labeled with [^{32}P]dATP and [^{32}P]dCTP by synthesis with the large fragment of DNA polymerase. Hybridization was performed at low stringency conditions: 40°C. in a solution containing 25.0% formamide, 5x SSC (1x SSC is 0.15M sodium chloride, 0.015M sodium

citrate), 1x Denhardt's solution (0.02% polyvinylpyrrolindone, 0.02% Ficoll, 0.02% bovine serum albumin), and 25 µg/µl sonicated salmon sperm DNA. The filters were washed at 40°C. in 0.1x SSC containing 0.1% sodium dodecyl sulfate and exposed at -70°C. to Kodak XAR film in the presence of an intensifying screen. Lambda phage clones hybridizing with the probes were plaque purified and DNA was prepared for Southern blot analysis (Southern, 1975; Sambrook et al., 1989). A Genomic clone hybridizing with all five of the rat Y1 TM probes, designated hp25a, was isolated using this method. For subcloning and further Southern blot analysis, **the hp25a** DNA was cloned into pUC18 (Pharmacia, Piscataway, NJ). Nucleotide sequence analysis was accomplished by the Sanger dideoxy nucleotide chain termination method (Sanger et al., 1977) on denatured double-stranded plasmid templates, using Sequenase (US Biochemical Corp., Cleveland, OH).

Cloning and Sequencing of a rat NPY (Y4) neuropeptide receptor:

[0075] A rat spleen genomic library (Stratagene, La Jolla, CA) was screened using overlapping TM oligonucleotide probes (TM 1 - 7) derived from the nucleotide sequences corresponding approximately to the TM regions of the amino acid sequence of the human Y4 receptor as shown in Figure 2. The overlapping oligomers used were as follows:

TM1: nts. #129-201,
(+) strand/5'-TCATCGTCACTTCCTACAGCATTGAGACTGTCGTGG GGGTCCTGGGT (Sequence I.D. No.)and
(-) strand/5'-ACAGTCACACACATCAGGCAGAGGTTACCCAGGAC CCCCACGACAG (Seq. I.D. No.);
TM2: nts. #234-303,
(+) strand/5'-TGCTTATCGCCAACCTGGCCTTCTCTGACTTCCTCATGTGCCTCC (Seq. I.D. No.) and
(-) strand/5'-TAGACGGCGGTCAGCGGCTGGCAGAGGAGGCACATGAGGAAGTCA (Seq. I.D. No.);
TM3: nts. #348-417,
(+) strand/5'-TGTCGGCCTTCATCCAGTGCATGTCGGTGACGGTCTCCATCCTCT (Seq. I.D. No.) and
(-) strand/5'-CTCTCCAGGGCCACGAGGACGAGCGAGAGGATGGAGACCGTCACC (Seq. I.D. No.);
TM4: nts. #467-536,
(+) strand/5'-GCCTACCTGGGGATTGTGCTCATCTGGGTCATTGCCTGTGTCCTC (Seq. I.D. No.) and
(-) strand/5'-TGCTGTTGGCCAGGAAGGGCAGGGAGAGGACACAGGCAATGACCC (Seq. I.D. No.);
TM5: nts. #637-706,
(+) strand/5'-CATCTACACCACCTTCCTGCTCCTCTTCCAGTACTGCCTCCCACT (Seq. I.D. No.) and
(-) strand/5'-TGCATAACAGACCAGGATGAAGCCCAGTGGGAGGCAGTACTGGAA (Seq. I.D. No.);
TM6: nts. #800-870,
(+) strand/5'-CTGGTGGTGATGGTGGTGGCCTTTGCCGTGCTCT GGCTGCCTCTGC (Seq. I.D. No.) and
(-) strand/5'-CAGTCTTCCAGGCTGTTGAACACATGCAGAGGCAGCCAGAGCACG (Seq. I.D. No.);
TM7: NTS. #908-977,
(+) strand/5'-ATCTTCTTAGTGTGCCACTTGCTTGCCATGGCCTCCACCTGCGTC (Seq. I.D. No.) and
(-) strand/5'-TGAGAAAGCCATAGATGAATGGGTTGACGCAGGTGGAGGCCATGG (Seq. I.D. No.) were labeled with [^{32}P]-ATP and [^{32}P]-CTP by synthesis with the large fragment of DNA polymerase. Hybridization was performed at reduced stringency conditions: 40°C in a solution containing 37.5% formamide, 10% dextran sulfate, 5X SSC, 1x Denhardt's solution, and 100 µg/ml of sonicated salmon sperm DNA. The filters were washed at 45°C in 0.1X SSC containing 0.1% sodium dodecyl sulfate (SDS) and exposed at -70°C to Kodak XAR film in the presence of an intensifying screen. Lambda phage clones hybridizing to the probes were plaque purified by successive plating and rescreening. A genomic clone hybridizing with all seven human Y4 receptor TM probes, designated rs16b, was isolated using this method. For expression and sequence analysis, a 2.0 kb BamHI/HindIII fragment of rs16b was subcloned into the corresponding polylinker sites of a pcEXV-3 eukaryotic expression vector (Miller and Germain, 1986) modified to include a polylinker with EcoRI, SstI, ClaI, KpnI, SmaI, XbaI, BamHI, SalI and HindIII restriction sites and designated EXJ.RH. Nucleotide sequence analysis was accomplished by the Sanger dideoxy nucleotide chain-termination method (Sanger, 1977) on double stranded plasmid templates, using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio).

[0076] Transient Transfection: The entire coding region of hp25a (1127 bp), including 680 bp of 5' untranslated (5' UT) and 205 bp of 3' untranslated sequence (3' UT), was cloned into the BamHI and EcoRI sites of the polylinker-modified eukaryotic expression vector pCEXV-3 (Miller et al., 1986), called EXJ.HR (J.B., unpublished data). Monkey kidney cells (Cos-7) were transiently transfected with plasmid hp25a/EXJ (expression vector containing the hp25a receptor gene) using DEAE dextran methodology (reagents obtained from Specialty Media, Lavellette, NJ).

[0077] The plasmid rs16b/EXJ (the expression vector containing the rs16b receptor gene), was transiently transfected into Cos-7 cells using similar methods, as were the human Y1 receptor (Larhammar, 1992) and the human Y2 receptor. The cloned Y2 receptor was disclosed in U.S. patent application 08/192,288 filed on February 2, 1994, currently pend-

ing, the foregoing contents of which are hereby incorporated by reference.

Stable Transfection

[0078] Human Y4 receptors were co-transfected with a G-418 resistant gene into the mouse embryonic NIH-3T3 cell line by a calcium phosphate transfection method (Cullen, 1987). Stably transfected cells were selected with G-418. Human Y4 receptors were similarly transfected into mouse fibroblast LM(tk-) cells.

[0079] Cell culture: COS-7 cells were grown on 150 mm plates (Corning) in D-MEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 2 mM glutamine, 100 units/ml penicillin/80 units/ml streptomycin) at 37 °C, 5% CO_2. Stock plates of COS-7 cells were trypsinized and split 1:6 every 3-4 days. SK-N-Be(2) human neuroblastoma cells were grown similarly in 225 cm^2 flasks (Co-star) using 50% Eagle's Modified Essential Media, 50% Ham's Nutrient Mixture F-12, 15% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin/80 units/ml streptomycin, and 1% non-essential amino acids. Stock flasks of SK-N-Be(2) cells were trypsinized and split 1:10 every 7 days.

[0080] Mouse embryonic NIH-3T3 cells were grown on 150 mm plates in Dulbecco's Modified Eagle Medium (DMEM) with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 µg/ml streptomycin) at 37 °C, 5% CO_2. Stock plates of NIH-3T3 cells were trypsinized and split 1:15 every 3-4 days. Mouse fibroblast LM(tk-) cells were grown on 150 mm plates in Dulbecco's Modified Eagle Medium (DMEM) with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 µg/ml streptomycin) at 37 °C, 5% CO_2. Stock plates of LM(tk-) cells were trypsinized and split 1:10 every 3-4 days.

[0081] Cell culture media and supplements were from Specialty Media (Lavallette, NJ). Cell culture plates (150 mm) were from Corning (Corning, NY). Cell culture flasks (225 cm^2) and polypropylene microtiter plates were from Co-star (Cambridge, MA).

[0082] Membrane Harvest: Membranes were harvested from COS-7 cells 48 hours after transfection and from SK-N-Be(2) seven days after splitting. Adherent cells were washed twice in ice-cold phosphate buffered saline (138 mM NaCl, 8.1 mM Na_2HPO_4, 2.5 mM KCl, 1.2 mM KH_2PO_4, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, pH 7.4) and lysed by sonication in ice-cold hypotonic buffer (20 mM Tris-HCl, 5 mM EDTA, pH 7.7). Large particles and debris were cleared by low speed centrifugation (200 x g, 10 min, 4 °C). Membranes were collected from the supernatant fraction by high speed centrifugation (32,000 x g, 18 min, 4 °C), washed with ice-cold hypotonic buffer, and collected again by high speed centrifugation (32,000 x g, 18 min, 4 °C). The final membrane pellet was resuspended by sonication into a small volume (~500 µl) of ice-cold binding buffer (10 mM NaCl, 20 mM HEPES, 0.22 mM KH_2PO_4, 1.26 mM $CaCl_2$, 0.81 mM $MgSO_4$, pH 7.4). Protein concentration was measured by the Bradford method (Bradford, 1976) using Bio-Rad Reagent, with bovine serum albumin as a standard.

[0083] Radioligand Binding to Membrane Suspensions: Membrane suspensions were diluted in binding buffer supplemented with 0.1% bovine serum albumin and 0.1% bacitracin to yield an optimal membrane protein concentration: ~ 0.02 mg/ml for human Y1 receptors, ~ 0.015 mg/ml for hp25a receptors, and ~ 0.25 mg/ml for SK-N-Be(2). (Under these conditions, ^{125}I-PYY bound by membranes in the assay was less than 10% of ^{125}I-PYY delivered to the sample.) ^{125}I-PYY and non-labeled peptide competitors were also diluted to desired concentrations in supplemented binding buffer. Individual samples were then prepared in 96-well polypropylene microtiter plates by mixing membrane suspensions (200 ul), ^{125}I-PYY (25 ul), and non-labeled peptides or supplemented binding buffer (25 ul). Samples were incubated in a 30 °C water bath with constant shaking for 120 min. Incubations were terminated by filtration over Whatman GE/C filters (pre-coated with 0.5% polyethyleneimine and air-dried before use). Filter-trapped membranes were counted for ^{125}I in a gamma counter. Non-specific binding was defined by 100 nM human PP for hp25a receptors and by 100 nM NPY for Y1 and SK-N-Be(2) receptors. Specific binding in time course and competition studies was typically 80%; most non-specific binding was associated with the filter. Binding data were analyzed using nonlinear regression and statistical techniques available in the GraphPAD InPlot package (San Diego, CA). Porcine ^{125}I-PYY was from New England Nuclear (Boston, MA). NPY and related peptide analogs were from either Bachem California (Torrance, CA) or Peninsula (Belmont, CA). Whatman GE/C filters were from Brandel (Gaithersburg, MD). Bio-Rad Reagent was from Bio-Rad (Hercules, CA). Bovine serum albumin and bacitracin were from Sigma (St. Louis. MO). All other materials were reagent grade. Functional Assay: Radioimmunoassay of cAMP Stably transfected cells were seeded into 96-well microtiter plates and cultured until confluent. To reduce the potential for receptor desensitization, the serum component of the media was reduced to 1.5% for 4 to 16 hours before the assay. Cells were washed in Hank's buffered saline, or HBS (150 mM NaCl, 20 mM HEPES, 1 mM $CaCl_2$, 5 mM KCl, 1 mM $MgCl_2$, and 10 mM glucose) supplemented with 0.1% bovine serum albumin plus 5 mM theophylline and pre-equilibrated in the same solution for 20 mm at 37 °C in 5% CO_2. Cells were then incubated 5 mm with 10 µM forskolin and various concentrations of receptor-selective ligands. The assay was terminated by the removal of HBS and acidification of the cells with 100 mM HCl. Intracellular cAMP was extracted and quantified with a modified version of a magnetic bead-based radioimmunoassay (Advanced Magnetics, Cambridge, MA). The final antigen/antibody complex was separated from free ^{125}I-cAMP by vacuum filtration through a PVDF filter in a microtiter plate (Millipore, Bedford, MA). Filters were punched and counted for ^{125}I in a Packard

gamma counter. Binding data were analyzed using nonlinear regression and statistical techniques available in the GraphPAD Prism package (San Diego, CA).

[0084] Functional Assay: Intracellular Calcium Mobilization The intracellular free calcium concentration was measured by microspectroflourometry using the fluorescent indicator dye Fura-2/AM. Stably transfected cells were seeded onto a 35 mm culture dish containing a glass coverslip insert. Cells were washed with HBS and then loaded with 100 µl of Fura-2/AM (10 µM) for 20 to 40 min. After washing with HBS to remove the Fura-2/AM solution, cells were equilibrated in HBS for 10 to 20 min. Cells were then visualized under the 40X objective of a Leitz Fluovert FS microscope and fluorescence emission was determined at 510 nM with excitation wave lengths alternating between 340 and 380 nM. Raw fluorescence data were converted to calcium concentrations using standard calcium concentration curves and software analysis techniques.

Tissue Localization and Gene Expression: Reverse Transcriptase PCR

[0085] Human tissues (obtained from National Disease Research Interchange) were homogenized and total RNA extracted using guanidine isothiocyanate/CsCl cushion method (Kingston, 1987). RNA was treated with DNase to remove any contaminating genomic DNA. cDNA was prepared from total RNA with random hexanucleotide primers using reverse transcriptase (Superscript II; BRL). An aliquot of the first strand cDNA (250ng of total RNA) was amplified in a 50 µl PCR reaction mixture (200µM dNTPs final concentration) containing 1.2U of Taq polymerase in the buffer supplied by the manufacturer (Perkin-Elmer Corporation), and 1 µM of primers, using a program consisting of 30 cycles of 94°C./2', 68°C./2', and 72°C./3', with a pre- and post-incubation of 95°C./5' and 72°C./10', respectively. PCR primers for human Y4 were designed against the human Y4 sequence in the third intracellular loop and carboxy terminal regions: 5'-CGCGTGTTTCACAAGGGCACCTA-3' and 5'-TGCCACTTAGCCTCAGGGACCC-3', respectively.

[0086] The PCR products were run on a 1.5% agarose gel and transferred to charged nylon membranes (Zetaprobe GT, BioRad), and analyzed as Southern blots. Hybridization probes corresponding to the receptor region flanked by PCR primers were prepared (5'-TCCGTATGTACTGTGGACAGGGGCAGATGCTCCGACTCCTCCAGG-3') and prescreened for the absence of cross-reactivity with human Y1 and human Y2 receptor subtypes. Filters were hybridized with end-labeled [γ-^{32}P]ATP internal probe to the PCR primers, washed under high stringency, and exposed to Kodak XAR film in the presence of an intensifying screen, as described above. Similar PCR and Southern blot analysis were conducted with primers and probe directed to the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (Clontech, Palo Alto, CA), and demonstrated that equal amounts of cDNA from the different tissues were being assayed for NPY expression.

Results

[0087] A human genomic placenta library was screened, under reduced stringency conditions, with oligonucleotide probes directed to the first, second, third, fifth, and seventh transmembrane regions of the rat Y1 neuropeptide receptor gene (Eva, C. et al., 1990; GenBank accession No. Z11504) . Positively-hybridizing clones (≈100-150) were isolated, plaque-purified and characterized by Southern blot analysis and sequencing. One clone, hp25a, contained a 1.3 kb PstI fragment which hybridized with the rat Y1-derived oligonucleotide probes and was subsequently subcloned into a pUC vector. DNA sequence analysis indicated greatest homology to the rat and human Y1 receptor genes. This clone was a partial intronless gene fragment, encoding part of the third intracellular loop through the carboxyl terminus, including a termination codon.

[0088] In order to obtain a full-length clone, a 2.0 kb BamHI/EcoRI hybridizing fragment, containing the entire coding region, which was intronless, was subcloned into an expression vector and sequenced. The genomic full-length construct in the expression vector (called hp25a/EXJ) contains an open reading frame of 1127 bp, with 680 bp of the predicted 5' UT and 205 bp of predicted 3' UT sequence, and encodes a protein of 375 aa in length, with a relative molecular mass of ≈41,000 daltons. Hydropathy analysis of the protein is consistent with a putative topography of seven transmembrane domains, indicative of the G protein-coupled receptor family.

[0089] Initial sequence analysis revealed that clone hp25a/EXJ contained several conserved structural features/residues found among the members of the neuropeptide receptor family, including two glycines and asparagine in TM1 (positions 55, 58 and 59, respectively, in Fig. 2), an asparagine, leucine and aspartic acid in TM2 (positions 82, 83, and 87, respectively, in Fig. 2), a serine and leucine in TM3 (positions 128 and 132, respectively, in Fig. 2), a tryptophan and proline in TM4 (positions 164 and 173, respectively, in Fig. 2), a tyrosine and proline in TM5 (positions 223 and 226, respectively, in Fig. 2), a phenylalanine, tryptophan, and proline in TM6 (positions 275, 279, and 281, respectively, in Fig. 2), and a serine, threonine, asparagine, and proline in TM7 (positions 315, 316, 319, and 320, respectively, in Fig. 2). Other features of this human hp25a receptor gene are the presence of three potential sites for N-linked glycosylation in the amino terminus (asparagine residues 2, 19, and 29; Fig. 1) and the presence of several serines and threonines in the carboxyl terminus and intracellular loops, which may serve as sites for potential phosphorylation by protein

kinases.

[0090] A comparison of nucleotide and peptide sequences of clone hp25a/EXJ with sequences contained in the Genbank/EMBL databases reveals that the clone is most related to the rat, mouse and human Y1 receptor genes and proteins (see Fig. 2). The hp25a clone exhibits 42% overall amino acid identity with the human NPY-1 receptor and 55% identity when comparing only the transmembrane domains between hp25a and Y1. The comparison of the individual amino acid residues in the TM domains between hp25a and Y1 reveal <30%, 57%, 57%, 57%, 52%, 63%, and 71% identity in the corresponding one through seven TM regions, repectively. The hp25a clone hybridized only with the TM7-specific probe from the original set of rat-derived TM probes originally used to screen the library which is consistent with the hp25a clone sharing the highest degree of amino acid identity with the TM7 domain of the rat Y1 receptor.

[0091] A rat homolog of the human Y4 receptor, designated rs16b, was isolated from a rat spleen genomic library using probes derived from the transmembrane regions of the human Y4 receptor. The nucleotide sequence of rs16b is 80% identical in the coding region to the nucleotide sequence of the human Y4 receptor, and encodes a protein 375 amino acids in length (Figure 3). The rs16b clone exhibits 75% overall amino acid identity with the human Y4 amino acid sequence, and in the putative transmembrane domains (TMs), the protein predicted by rs16b exhibits 84% amino acid identity with the human Y4 receptor. This degree of primary amino acid sequence identity is lower than is typically seen for species homologues, and suggests that rat and human Y4 receptors may exhibit functional variations as well. The predicted intracellular loop between TMs V and VI is particularly divergent, showing only 56% amino acid identity between rat and human Y4; divergence in this region could potentially mediate differences in G-protein coupling between the rat and human receptors. The primary sequences of rat and human Y4 receptors also show differences in their patterns of sequence motifs for casein kinase II phosphorylation, N-myristoylation, and protein kinase C phosphorylation; these sites could potentially mediate differences in the function or regulation of the two receptors.

[0092] Monkey kidney cells transiently expressing the gene encoding the hp25a receptor were used for pharmacological evaluation. Membranes harvested from transiently transfected Cos-7 cells exhibited high affinity, saturable [^{125}I]PYY binding. The time course of specific binding was measured in the presence of 0.06 nM ^{125}I-PYY (Fig. 5). The association curve was monophasic, with a an observed association rate (K_{obs}) of 0.12 ± 0.02 min^{-1} and a $t_{1/2}$ of 6 min; equilibrium binding was 95% complete within 26 min and 100% complete within 50 min (n = 3). For comparison, we also measured the time course of binding to human Y1 receptors transiently expressed in COS-7 cells. The association curve was monophasic, with a K_{obs} of 0.06 ± 0.02 min^{-1} and a $t_{1/2}$ of 12 min; equilibrium binding was 95% complete within 51 min and 100% complete within 90 min (n = 3) (data not shown). The different patterns of radioligand association for hp25a and human Y1 receptors suggest novel mechanisms of receptor/ligand interaction.

[0093] Saturation binding data for ^{125}I-PYY were fit to a one-site model with an apparent K_d of 0.11 ± 0.01 nM and an apparent B_{max} of 1.42 ± 0.05 pmol/mg membrane protein, corresponding to approximately 1.4 x 10^5 receptors/cell (n = 4; Fig. 6). Given that the transfection efficiency was 20-30% (data not shown), the receptor density on transfected cells was probably closer to 7 x 10^5/cell. Membranes from mock-transfected cells, when prepared and analyzed in the same way as those from hp25a-transfected cells, displayed no specific binding of ^{125}I-PYY. We conclude that the ^{125}I-PYY binding sites observed under the described conditions were derived from the hp25a construct.

[0094] The pharmacological profile of hp25a was defined by membrane binding assays. The receptor was probed for features of all well characterized pancreatic polypeptide family receptors including Y1, Y2, Y3, and PP. The rank order of affinity for several peptide analogs was derived from competitive displacement of ^{125}I-PYY (Fig. 7 and Table 2). The hp25a receptor was compared with two model systems: 1) the cloned human Y1 receptor (Larhammar et al., 1992; Herzog et al., 1992) transiently expressed in COS-7 cells, and 2) the Y2-like receptor population expressed by human SK-N-Be(2) neuroblastoma cells (Wahlestedt et al., 1991; Dumont et al., 1992). No models for human Y3 and human PP receptors have been described.

[0095] PP bound to hp25a with extremely high affinity (K_i = 0.029 nM) and dramatic selectivity: PP was > 6000-fold selective for hp25a over human Y1 receptors (K_i = 200 nM) and SK-N-Be(2) receptors (K_i > 300 nM). This profile suggests that hp25a could function selectively as a PP receptor *in vivo*. The data further indicated, however, that hp25a bound quite well to human NPY (K_i = 1.4 nM) and even better to human PYY (K_i = 0.62 nM). These K_i values, while lower than the K_i for PP, are comparable to the effective concentrations of NPY and PYY from numerous physiological and pharmacological studies (Dumont, 1992). In our investigation, SK-N-Be(2) receptors bound human NPY and human PYY in the same rank order as hp25a but with 5- to 10-fold higher affinity, whereas human Y1 receptors bound human NPY and human PYY in the opposite rank order with 5- to 30-fold higher affinity. Hydrolysis of the carboxy terminal amide to free carboxylic acid, as in human NPY free acid, was disruptive for binding to all receptors. A requirement for a carboxy terminal amide appears to be a common structural feature of all pancreatic polypeptide family peptide/receptor interactions.

[0096] Fuhlendorff and co-workers replaced Ile^{31} and Gln^{34} in NPY with the corresponding residues from PP to create [Leu^{31},Pro^{34}]NPY, which is commonly used to distinguish Y1 from Y2 receptors (Fuhlendorff, 1990). Human [Leu^{31},Pro^{34})NPY displayed > 2300-fold selectivity for human Y1 receptors over SK-N-Be(2), but only 5-fold selectivity for human Y1 receptors over hp25a. Human [Leu^{31},Pro^{34}]NPY was a better ligand for hp25a (K_i = 0.60 nM) than was

human NPY itself (K_i = 1.4 nM). This is possibly a reflection of the way in which [Leu^{31},Pro^{34}]NPY mimics PP at positions 31 and 34. In contrast, the [Leu^{31},Pro^{34}]NPY analog was well tolerated by the human Y1 receptor (K_i = 0.13 nM), but not preferred over the parent peptide (K_i = 0.049 nM).

[0097] hp25a displayed an intermediate level of sensitivity to N-terminal deletions of NPY and PYY, less so than human Y1 receptors. Removing Tyr^1 from porcine NPY resulted in a 29-fold loss in affinity for human Y1 receptors when compared with the full length parent peptide. The same modification decreased affinity 4-fold for hp25a receptors and 3-fold for SK-N-Be(2) receptors. It is interesting in this regard that human PP contains Ala^1; the Tyr^1 of NPY may not play much of a role in receptor recognition. Truncation to NPY_{13-36} decreased affinity 1000-fold for human Y1 receptors, 33-fold for hp25a, and 4-fold for SK-N-Be(2) receptors. Further truncation to porcine NPY_{22-36} decreased affinity 3500-fold for human Y1 receptors, 120-fold for hp25a, and 11-fold for SK-N-Be(2) receptors. In this regard, the hp25a receptor shares features of both Y1- and Y2-like pharmacology, as would be expected if the N-terminal region of porcine NPY were only moderately involved in receptor recognition.

[0098] An important structural difference between human PP, human PYY and human NPY is that both human NPY and PYY contain Gln^{34}, whereas human PP contains Pro^{34}. When Gln^{34} in NPY was replaced with Pro^{34} (as in the analog [Leu^{31}, Pro^{34}]NPY), an increase in binding affinity for the human Y4 receptor was observed. A similar increase in binding affinity was detected when Gln^{34} of PYY was replaced with Pro^{34}, supporting the proposal that PP-like peptides are preferred by the Y4 receptor. Replacement of Pro^{34} in human PP by Gln^{34} (as in [Ile^{31}, Gln^{34}]PP) caused very little change in PP binding affinity, however, suggesting that in the case of PP there are significant contributions to binding affinity from other regions of the peptide structure.

[0099] Applicants further extended the structure/activity data for human PP fragments (PP_{2-36}, PP_{13-36}, PP_{20-36}, PP_{27-36}, and PP_{31-36}). PP binding was unaffected by N-terminal truncation to PP_{2-36}, but further truncation to PP_{13-36} and beyond was disruptive. The shortest PP fragment tested, PP_{31-36}, bound selectively to the Y4 receptor with K_i = 350 nM, and hydrolysis of the C-terminal amide was detrimental (K_i > 10,000 nM for human PP_{31-36} free acid), as reported earlier for NPY. We conclude that the binding of PP to the Y4 receptor resembles the binding of NPY to the Y1 receptor, in that 1) Pro^{34} is well-tolerated and 2) both ends of the peptide are required for optimal binding activity. This is in contrast to the Y2 binding model, in which 1) Pro^{34} is not well-tolerated and 2) the N-terminal region of NPY does not contribute significantly to binding affinity. Note also that the Y2-selective ligands human PYY_{3-36} and C2-NPY display relatively low affinity for the human Y4 receptor.

[0100] Additionally, the binding of the tetrapeptide invertebrate neurotransmitter Phe-Met-Arg-Phe-Amide (FMRF-amide) was investigated. This peptide has been shown to mimic several functions of NPY including the stimulation of food intake in rats (Robert, 1988). FMRFamide bound selectively to the Y4 receptor with a K_i value of 4000 nM. A closely related derivative, Phe-Leu-Arg-Phe-amide (FLRFamide), displayed improved Y4 binding affinity (K_i = 750 nM) while maintaining selectivity. We also investigated the binding of [D-Trp^{32}]NPY. This peptide was reported to stimulate food intake when injected into rat hypothalamus, and also to attenuate NPY-induced feeding in the same paradigm (Balasubramaniam, 1994). [D-Trp^{32}]NPY displayed relatively low binding affinity for the human Y4 receptor as well as for the human Y1 and Y2 receptor subtypes. Data for these and other new peptides not included in the original patent filing are listed in Table 3.

[0101] Untransfected NIH-3T3 and LM(tk-) were pre-screened for specific ^{125}I-PYY binding and found to be negative (data not shown). After co-transfection with the human Y4 cDNA and a G418-resistant gene and selection with G-418, surviving colonies were screened for specific binding of ^{125}I-PYY. Two positive clones were identified and isolated for further study (NIH-3T3 hY4 clone #5 and LM(tk-) hY4 clone #3). The binding of ^{125}I-PYY to membranes from the NIH-3T3 stable clone was saturable over a radioligand concentration range of 0.5 pM to 2.5 nM. Binding data were fit to a one-site binding model with an apparent K_d of 0.17 nM ± 0.005 and a receptor density of 350 ± 80 fmol/mg membrane protein (mean ± s.e.m., n = 2). The LM(tk-) clone displayed an estimated receptor density of 7 fmol/mg membrane protein during the primary selection screen and was not analyzed further in a saturation assay.

[0102] Activation of all Y-type receptors described thus far is thought to involve coupling to pertussis toxin-sensitive G-proteins which are inhibitory for adenylate cyclase activity (G_i or G_o) (Wahlestedt and Reis, 1993). Based on these prior observations, we investigated the ability of PP to inhibit forskolin-stimulated cAMP accumulation in LM(tk-) cells stably expressing the human Y4 receptor. Incubation of intact cells with 10 µM forskolin produced ~10-fold increase in cAMP accumulation over a 5 minute period, as determined by radioimmunoassay. Simultaneous incubation with human PP decreased the forskolin-stimulated cAMP accumulation by 67% in stably transfected LM(tk-) cells (Fig. 8) but not in untransfected cells (data not shown). Applicants conclude that human Y4 receptor activation can result in decreased cAMP accumulation, very likely through inhibition of adenylate cyclase activity.

[0103] Peptides selected for their ability to bind to the transiently expressed human Y4 receptor were investigated for their ability to activate the human Y4 in the cAMP assay (Table 4). Note that both human PP and human PP_{2-36} bound the Y4 receptor with a K_i value of 0.06 nM, and that each displayed comparable activity in the cAMP assay with closely matching EC_{50} values of 0.09 nM and 0.08 nM, respectively. The truncated PP fragments PP_{27-36} and PP_{31-36} were relatively weak ligands in the binding assay and were also less than 50% as effective as the full length PP in reducing

forskolin-stimulated cAMP, thereby acting as partial agonists. Similarly, both NPY and PYY (which deviate from PP primarily in the N-terminal regions) yielded EC_{50} values ≥ 10-fold larger than their K_i values. Receptor activation (more so than binding) may therefore depend heavily upon N-terminal PP structure. The functional activity of the reported feeding behavior modulator [D-Trp^{32}]NPY was also investigated. Consistent with this peptide's low binding affinity for the human Y4 receptor, no functional activity of the peptide was detected at concentrations up to 0.3 uM (see Table 4), or when tested at 0.3 uM for antagonism of the PP functional response (data not shown).

[0104] The intracellular free calcium concentration was markedly increased in LM(tk-) cells stably transfected with the human Y4 receptor after application of 100 nM human PP (Δ $[Ca^{2+}]_i$ = 325 nM; Fig. 9). The response to 100 nM NPY was relatively small (Δ $[Ca^{2+}]_i$ = 68 nM). Untransfected LM(tk-) cells were unresponsive to either peptide (data not shown). When human PP was further analyzed in a concentration/response curve, the maximum Δ $[Ca^{2+}]_i$ measured was 334 nM and the EC_{50} was 35 nM (Fig. 9, Inset). This greater activity of PP over NPY is consistent with the pharmacological profiles derived from both binding and cAMP assays described above. The calcium mobilization assay thereby provides a second pathway through which Y4 receptor activation can be measured.

[0105] Y4 mRNA was detected by PCR techniques in a broad range of human tissues. Relatively intense hybridization signals were detected in total brain, coronary artery, and ileum, suggesting a potential role for Y4 receptors in CNS function, cardiovascular regulation, and gastrointestinal physiology (Table 5).

[0106] The cDNA corresponding to the rat Y4 homolog was transiently expressed in COS-7 cells for membrane binding studies. The binding of ^{125}I-PYY to the rat Y4 receptor was saturable over a radioligand concentration of 0.5 pM to 2.5 nM. Binding data were fit to a one-site model with an apparent K_d of 0.15 nM ± 0.005 and a receptor density of 275 ± 3 fmol/mg membrane protein (mean ± s.e.m., n = 2). As determined by using peptide analogs within the pancreatic polypeptide family, the rat Y4 pharmacological profile bears a resemblance to the human Y4 receptor; there are several interesting exceptions, however, including frog PP, salmon PP, human PP_{31-36}, and avian PP, each of which discriminated ~10-fold between the rat and human receptor subtypes (Table 6). The differences may reflect the fact that PP is not well conserved among species relative to NPY and PYY; hence the species homologs of PP are likely to exhibit more variability in ligand binding.

[0107] In summary, both the human Y4 receptor and the rat Y4 receptor displayed features unique among the neuropeptide receptors, exhibiting a profile which is divergent from their closest relatives, Y1 or Y2, in that each binds optimally to PP rather than to NPY or PYY (see Tables 1, 2 and 6). Unlike the Y1 and Y2 receptor models, the Y4 receptor appears to be a reasonable target for all three peptide ligands.

TABLE I

Pharmacologically defined receptors for NPY and related pancreatic polypeptides.

Rank orders of affinity are based on published reports of binding and functional data (Wahlestedt et al., 1991; Schwartz et al., 1990; Wahlestedt et al., 1993; Dumont et al., 1992). Missing peptides in the series reflect a lack of published information.

Receptor	Affinity (-pK_i or -pEC_{50})					
	11 to 10	10 to 9	9 to 8	8 to 7	7 to 6	< 6
Y1	NPY PYY [Leu^{31},Pro^{34}]NPY		NPY_{2-36}	NPY_{13-36}	PP	
Y2		PYY NPY NPY_{2-36}	NPY_{13-36}			[Leu^{31},Pro^{34}]NPY PP
Y3		NPY	[Pro^{34}]NPY	NPY_{13-36} PP		PYY
PP	PP		[Leu^{31},Pro^{34}]NPY			NPY

TABLE 2

Pharmacological profile of the hp25a receptor.

Binding data reflect competitive displacement of ^{125}I-PYY from membranes of COS-7 cells transiently expressing hp25a receptors. Peptides were tested at concentrations ranging from 0.001 nM to 100 nM. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to K_i values according to the equation, $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the ^{125}I-PYY concentration and K_d is the equilibrium dissociation constant of ^{125}I-PYY. The data shown are representative of at least two independent experiments.

Competitor	Human Y1, K_1 (nM)	hp25a, K_i (nM)	SK-N-Be(2), K_i (nM)
human PP	200 ± 68	0.029 ± 0.006	> 300
human [Leu^{31,Pro}34]NPY	0.13 ± 0.02	0.60 ± 0.09	> 300
human PPY	0.085 ± 0.021	0.62 ± 0.15	0.11 ± 0.02
porcine NPY	0.049 ± 0.001	1.2 ± 0.2	0.28 ± 0.04
human NPY	0.049 ± 0.009	1.4 ± 0.1	0.13 ± 0.02
porcine NPY_{2-36}	1.4 ± 0.2	4.4 ± 1.3	0.41 ± 0.09
porcine NPY_{13-36}	51 ± 16	39 ± 5	1.8 ± 0.4
porcine PYY_{13-36}	32 ± 7	47 ± 6	0.86 ± 0.14
porcine NPY_{16-36}	45 ± 4	54 ± 2	5.0 ± 0.5
porcine NPY_{18-36}	28 ± 5	63 ± 7	2.1 ± 0.5
human NPY free acid	> 300	79 ± 17	280 ± 120
porcine NPY_{20-36}	62 ± 6	100 ± 20	3.1 ± 0.6
porcine NPY_{22-36}	170 ± 30	140 ± 63	3.2 ± 0.6
porcine NPY_{26-36}	> 300	> 300	70 ± 7

Table 3: human Y4 receptor vs. Y-type receptors cloned from human.

[0108] Binding data reflect competitive displacement of ^{125}I-PYY from membranes of COS-7 cells transiently expressing human Y1, human Y2, and human Y4 receptors. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to K_i values according to the equation Chang-Prusoff equation, $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the ^{125}I-PYY concentration and K_d is the equilibrium dissociation constant of ^{125}I-PYY. Any peptide not included in the original patent filing is referred to as a "new peptide".

Table 3

Peptide	Y1	Y2	Y4	Comments
PP, human	77	> 1000	**0.06**	
PP_{2-36}, human	> 40	> 100	**0.06**	new peptide
PP_{13-36}, human	> 100	> 100	**39**	new peptide
PP_{20-36}, human	> 100	> 100	**> 100**	new peptide
PP_{27-36}, human	> 100	> 100	**> 88**	new peptide
PP_{31-36}, human	> 10000	> 10000	**350**	new peptide
PP_{31-36} free acid, human	> 10000	> 10000	**> 10000**	new peptide
Phe-Met-Arg-Phe-Amide	12000	75000	**4000**	
Phe-Leu-Arg-Phe-Amide	15000	> 100000	**750**	new peptide
[Ile^{31}, Gln^{34}]PP, human	> 86	20	**0.09**	new peptide
PP, bovine	240	> 820	**0.05**	new peptide
PP, rat	460	> 1000	**0.18**	new peptide

Table 3 continued

Peptide	Y1	Y2	Y4	Comments
PP, salmon	0.20	0.17	**3.2**	new peptide
PP, avian	400	> 1000	**7.0**	new peptide
PP, frog	98	> 1000	**61**	new peptide
PYY, human	0.19	0.36	**0.87**	
PYY, porcine	0.14	0.35	**1.3**	new peptide
PYY_{3-36}, human	45	0.70	**14**	new peptide
PYY_{13-36}, porcine	33	1.5	**46**	
[Pro^{34}]PYY, human	0.14	> 310	**0.12**	new peptide
Peptide	Y1	Y2	**Y4**	Notes
NPY, human	0.08	0.74	**2.2**	
NPY, porcine	0.07	0.81	**1.1**	
Melanostatin (frog NPY)	0.07	0.87	**1.2**	new peptide
NPY_{2-36}, human	3.6	2.0	**16**	new peptide
NPY_{2-36}, porcine	2.4	1.2	**5.6**	
NPY_{13-36}, porcine	70	2.5	**38**	

Table 3 Continued

Peptide	Y1	Y2	Y4	Comments
NPY_{16-36}, porcine	41	3.6	54	
NPY_{18-36}, porcine	70	4.2	> 290	
NPY_{20-36}, porcine	63	3.6	120	
NPY_{22-36}, porcine	> 1000	18	> 990	
NPY_{26-36}, porcine	> 1000	380	304	
[Leu^{31}, Pro^{34}]NPY, human	0.15	> 120	1.1	
[Leu^{31}, Pro^{34}]NPY, porcine	0.15	> 540	1.5	new peptide
O-Me-Tyr^{21}-NPY, human	0.12	1.55	6.1	new peptide
NPY free acid, human	490	> 1000	> 1000	
NPY_{1-24} amide, human	> 1000	> 1000	> 1000	new peptide
C2-NPY, porcine	73	3.5	120	new peptide
[D-Trp^{32}]NPY, human	> 1000	> 1000	> 1000	new peptide

TABLE 4: Functional activation of the human Y4 receptor and inhibition of cAMP accumulation.

[0109] K_i values were derived from binding assays as described in Table 3. Peptides were evaluated for binding affinity and then analyzed for functional activity. Functional data were derived from radioimmunoassay of cAMP accumulation in stably transfected LM(tk-) cells stimulated with 10 μM forskolin. The maximum inhibition of cAMP accumulation relative to that produced by human PP (E_{max}) and the concentration producing a half-maximal effect (EC_{50}) were determined by nonlinear regression.

Table 4

Peptide	Binding	Function	
	K_i(nM)	EC_{50} (nM)	E_{max}
PP, human	0.06	0.09	100%
PP_{2-36}, human	0.06	0.08	101%
PP_{13-36}, human	39	580	96%
PP_{27-36}, human	> 88	3500	50 %
PP_{31-36}, human	> 10000	89000	47 %
[Ile^{31},Gln^{34}]PP , human	0.09	0.27	101%
salmon PP	3.2	110	96%
PYY, human	0.87	47	118%
[Pro^{34}]PYY, human	0.12	1.1	106%
NPY, human	2.2	20	98%
NPY, porcine	1.1	68	105%
NPY_{18-36}	> 290	Not detected	
[Leu^{31},Pro^{34}]N PY, human	1.1	35	105%
[Leu^{31},Pro^{34}]N PY,porcine	1.5	26	111%
[D-Trp^{32}]NPY, human	> 1000	Not detected	

TABLE 5: Macrolocalization of Y4 receptor mRNA in human tissues by PCR.

[0110] Localization data reflect PCR-based amplification of human Y4 cDNA derived from mRNA extracts of human tissues. Southern blots of the PCR products were prepared and hybridized with ^{32}P-labeled oligonucleotide probes selective for Y-type receptor subtypes. The labeled products were recorded on X-ray film and the relative signal density was determined by visual inspection. In this rating scheme, + = faint signal, + + = moderate signal, + + + = intense signal.

Table 5

Human tissues	human Y4 PCR product
total brain	+++
frontal brain	+
ventricle (heart)	+ +
atrium (heart)	+

Table 5 (continued)

Human tissues	human Y4 PCR product
thoracic aorta	+ +
coronary artery	+ + +
nasal mucosa	+
mesentery	+ +
stomach	+ +
ileum	+ + +
pancreas	not determined
liver	(-)
kidney	not determined
bladder	+
penis	+
testes	+
uterus (endometrium)	+ +
uterus (myometrium)	+

TABLE 6: Pharmacological binding profile of the rat Y4 receptor vs. the human Y4 receptor.

[0111] Binding data reflect competitive displacement of ^{125}I-PYY from membranes of COS-7 cells transiently expressing rat Y4 and human Y4 receptors. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to K_i values according to the equation Chang-Prusoff equation, $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the ^{125}I-PYY concentration and K_d is the equilibrium dissociation constant of ^{125}I-PYY.

Table 6

Peptide	**Rat Y4**	Human Y4
PP, human	**0.12**	0.06
PP, rat	**0.20**	0.18
PP, bovine	**0.15**	0.05
PP, frog	**0.19**	62
PP, salmon	**0.36**	3.2
PP_{31-36}, human	**20**	350
PP, avian	**> 82**	7
PP_{31-36} free acid, human	**> 100**	> 10000
PYY, porcine	**0.58**	1.3
NPY, human	**1.7**	2.2
NPY, porcine	**1.8**	1.1
NPY_{2-36}, human	**5**	16
NPY_{13-36}, porcine	**135**	38

Table 6 (continued)

Peptide	Rat Y4	Human Y4
[Leu^{31},Pro^{34}]NPY, human	**0.59**	1.2
NPY free acid, human	**> 1000**	> 1000
C2-NPY, porcine	**22**	120
[D-Trp32]NPY, human	**> 1000**	> 1000

Discussion

[0112] Applicants have cloned DNA representing a novel human neuropeptide Y/peptide YY/pancreatic polypeptide receptor (Y4) from human genomic DNA. Of all known G protein-coupled receptor sequences (EMBL/Genbank Data Base), the greatest homology was displayed between hp25a and the Y1 receptor genes (mouse–Eva et al., 1992; rat--Eva et al., 1990; and human–Larhammar et al., 1992). Comparison of the human hp25a deduced amino acid sequence with known G protein-coupled receptor sequences indicates the greatest concentration of identical amino acids to be in the transmembrane domains. In these TM regions, the percentage of identity for hp25a clone is 55% compared to human Y1, and less than 35% with other members of the peptide subfamily and other G protein-coupled receptor subfamilies. The alignment of this human hp25a sequence, relative to other G protein-coupled receptors or other members of the neuropeptide receptor subfamily, specifically human Y1, indicates a unique sequence, proving hp25a is a newly characterized receptor. The homology of hp25a to Y1 indicates that it is related to the NPY/PYY/PP family of receptors.

[0113] While the hp25a human receptor sequence exhibits higher overall and transmembrane identity to the rs16b rat Y4 receptor sequence than to other Y-type receptors such as the human Y1 receptor, the divergence between the rat Y4 and human Y4 sequences may contribute to the pharmacological differences between the two receptors. The isolation of the rat homologue of the Y4 receptor provides the means to compare the pharmacological properties of the rat and human Y4 receptors (see below) in relation to their observed differences in primary structures. These data will be critical to the design and testing of human therapeutic agents acting at these sites.

[0114] The unique pharmacological profile of the hp25a human Y4 receptor suggests that this receptor can serve as a novel target for the development of subtype selective ligands. The competitive displacement studies indicate that human PP is the preferred ligand for hp25a. The receptor also binds with high affinity to human NPY and human PYY, which share ≥ 47% amino acid identity with human PP. Affinity is enhanced by modifying NPY to closely resemble PP, as in [Leu^{31},Pro^{34}]NPY. Decreased affinity for C-terminal fragments of NPY suggest that both N- and C-terminal regions of NPY contribute to hp25a receptor recognition. hp25a was less sensitive to N-terminal deletion of NPY than was the human Y1 receptor. One may speculate that both Y1 and hp25a share a common mechanism of peptide interaction which has been optimized for either NPY or PP, respectively.

[0115] The pharmacological data do not support classification of hp25a as a Y1 receptor, in which case it would display > 4000-fold selectivity for binding to human NPY over human PP (Table 2). Neither do the data support classification as a Y2 receptor, in which case it would tolerate N-terminal deletion of NPY but not exchange of Gln^{34} for Pro^{34} (Table 2). Finally, the data fails to support the classification of hp25a as a Y3 receptor, since it would be expected to display greater affinity for NPY than for PP or PYY (Wahlestedt et al., 1991). Therefore, applicants are designating the hp25a receptor as a Y4 receptor.

[0116] The additional data included here reflect an increased understanding of receptor ligand/interactions. Our further characterization of Y4 receptor pharmacology has indicated, for example, that the binding affinity for either human NPY (K_i = 2.2 nM) or human PYY ($_I$ = 0.87 nM) can be enhanced by conversion to human [^{Leg}, Pro^{34}]NPY (K_i = 1.1. nM) or human [Pro^{34}]PYY (K_i = 0.14 nM). This information supports the importance of Pro34- in the peptide pharmacophore and could potentially be incorporated into the design of metabolically stable nonpeptide ligands with Y4-selectivity. Additionally, the data prompt a re-evaluation of literature reports in which [Pro^{34}]PYY is described as a Y1-selective ligand. Our results indicate that [Pro^{34}]PYY does not discriminate between the cloned human Y1 and cloned human Y4 receptor (K_i = 0.12 and 0.14 nM, respectively) such that [Pro^{34}]PYY cannot be used in isolation to define receptor subtypes.

[0117] Other particularly interesting peptides include FMRF-amide, FLRF-amide, and [D-Trp^{32}]NPY. FMRF-amide and [D-Trp^{32}]NPY have both been shown to modulate food intake in rats (get ref from George M). While FMRF-amide and its derivative displayed some degree of Y4-selectivity (albeit relatively low affinity compared to human PP), [D-Trp^{32}]NPY was essentially inactive at all Y-receptor subtypes studied. These profiles must be considered as efforts are undertaken to validate the receptor mechanism of NPY-induced food intake. The tetrapeptide FLRF-amide has additional value as a starting point for the design of small nonpeptide compounds with Y4 selectivity.

[0118] Applicants now have several Y4 receptor expression systems from which to chose, each uniquely suited to different research questions. The transient expression system in COS-7, for example, allows one to generate sufficient quantities of membranes for routine structure/activity relationship questions. Applicants can also produce mutant receptors by site-directed mutagenesis or other mutagenesis techniques and express them transiently in COS-7 for a comparison of pharmacological properties with those of the wild-type receptor. In this way, one can gain insight into receptor binding pockets, ligand binding domains, and mechanisms of activation. Whereas the transient expression system requires a new transfection for every cell or membrane harvest, the stable expression system offers the convenience of a single transfection step followed by routine passaging techniques. The stable system also offers the opportunity to select receptor density, which could be an important factor in evaluating the intrinsic activity of Y4 receptor ligands.

[0119] Applicants' characterization of the stably expressed Y4 receptor now shows definitively that the Y4 receptor can couple simultaneously to both cAMP regulation and calcium mobilization in a single cell type. The EC_{50} for the calcium response is significantly higher than the EC_{50} for the cAMP response, suggesting that calcium mobilization may reflect promiscuous coupling of the receptor to G-protein other than that required for cyclase regulation. The functional assays allow one to assign agonist and antagonist activities to receptor selective compounds and thereby provide one with critical tools for drug design.

[0120] The question logically arises as to whether hp25a should be classified as a PP receptor. To applicants' knowledge, no human PP receptor has been described. One must therefore look to the rat PP receptor for comparison. The rat PP receptor bound PP and analogs in the same rank order as hp25a (PP > [Leu^{31},Pro^{34}]NPY > NPY) (Schwartz et al., 1990). The rat PP receptor also appeared to bind both N- and C-terminal regions of the peptide ligand (Schwartz et al., 1987). A glaring discrepancy between hp25a and the rat PP receptor is that the latter displayed > 10,000-fold selectivity for PP over NPY (Schwartz et al., 1990).

In applicants' localization experiments Y4 mRNA was detected by PCR techniques in a broad range of human tissues. Relatively intense hybridization signals were detected in total brain, coronary artery, and ileum, suggesting a potential role for Y4 receptors in CNS function, cardiovascular regulation, and gastrointestinal physiology. This localization pattern is consistent with previously reported studies of PP-mediated effects at 1) brainstem sites (McTigue et al., 1993; Whitcomb et al., 1990), 2) on arterial blood pressure (Wager-Page et al., 1993a) and 3) on gastric acid secretion and gastrointestinal motility (McTigue et al, 1993; Wager-Page et al, 1993b. A more definitive localization of the Y4 receptor mRNA and receptor expression (i.e., whether on enterocytes, vascular smooth muscle cells, neurons, etc.) is attainable through *in situ* hybridization and receptor autoradiography techniques. There are to applicants' knowledge no published reports of PP receptor localization in human tissue as obtained through binding or functional studies. It may be informative, however, to compare the human Y4 macrolocalization data presented here with PP receptor characterization in the rat. PP receptors have been described, for example, in brainstem nuclei such as the area postrema, interpeduncular nucleus, dorsomedial nucleus, and the nucleus tractus solitarius (Whitcomb et al., 1990), consistent with the identification of Y4 mRNA in human brain. The PP receptors in rat brain stem are accessible to circulating PP, which is released upon vagal stimulation of the pancreas during feeding (Whitcomb et al., 1990). Activation of brainstem PP receptors inhibits further pancreatic secretion, increases gastric acid secretion, enhances gastric motility, and increases gastric emptying time (Louie et al., 1985; McTigue and Rogers, 1993). A Y4 receptor antagonist then, would be expected to slow down gastric emptying time and potentially reduce meal size.

[0121] Given the similarities in pharmacologic profiles between the published PP receptor and the hp25a human Y4 receptor, it would be tempting to call hp25a the human PP receptor. Applicants believe that calling hp25a the human PP receptor, however, would be misleading. This is because the relatively compressed window of affinity for PP. PYY, and NPY (0.02 nM $\leq K_i \leq$ 1.5 nM) makes hp25a a potential target for all three peptide ligands. Future localization experiments may help resolve the relationship between hp25a and the PP receptor.

[0122] Applicants propose that hp25a be known as the Y4 receptor. The name is not biased toward any one member of the pancreatic polypeptide family. The "Y" has its roots in the original classification of Y1 and Y2 receptor subtypes (Wahlestedt et al., 1987). The letter reflects the conservation in pancreatic polypeptide family members of the C-terminal tyrosine, described as "Y" in the single letter amino acid code. Applicants note that the cloned human Y1 receptor was introduced by Larhammar and co-workers as a "human neuropeptide Y/peptide YY receptor of the Y1 type", with peptide ligands listed in rank order of affinity (Larhammar et al., 1992). Similarly, hp25a could be described as a human pancreatic polypeptide/peptide YY/neuropeptide Y receptor of the Y4 type.

[0123] hp25a is to applicants' knowledge the first "Y type" receptor to be cloned from a subtype family other than Y1. The reported Y3 receptor cloned from bovine brain (Rimland et al., 1991) was later described as having been misidentified (Jazin et al., 1993; Herzog et al., 1993). A Y2-like receptor (PR4) was cloned from drosophila and characterized using mammalian analogs of NPY (Li et al., 1992); however, the classification of this receptor is controversial. The receptor was relatively insensitive to NPY; concentrations ranging from 0.3 to 10 µM were required to elicit calcium mobilization in oocytes injected with PR4 mRNA (Li et al., 1992). The receptor also displayed a rank order of potency for NPY analogs distinct from that observed in mammalian systems (Wahlestedt et al., 1993; Li et al., 1992). Furthermore, an NPY analog has not been isolated from drosophila (Wahlestedt et al., 1993). It is possible that an unidentified

ligand in drosophila can activate PR4 more readily than NPY, and as such, the receptor may eventually be reclassified.

[0124] The cloning and expression of a Y4 (hp25a) receptor represents a major advance in the ability to analyze numerous physiological processes mediated by the pancreatic polypeptide family. Binding sites for PP, PYY, or NPY have a widespread anatomical distribution in peripheral targets such as neuromuscular junction, smooth muscle, stomach chief cells, intestinal enterocytes, kidney proximal tubule, and fat cells (Dumont et al., 1992; Castan et al., 1992). These receptors are therefore in a position to potentially regulate a variety of physiological functions including cognition, circadian rhythm, EEG synchronization, body temperature, blood pressure, locomotor activity, neuroendocrine release, sexual/reproductive behavior, feeding, sympathetic activation, sensory transmission, gastrointestinal function, intestinal secretion, renal absorption, and cardiovascular function (Wahlestedt et al., 1993).

[0125] Y4 receptors are an invaluable resource for drug design. The pancreatic polypeptide family is potentially involved in several pathophysiological conditions including memory loss, depression, anxiety, epileptic seizure, pain, hypertension, locomotor problems, circadian rhythm disorders, eating/body weight disorders, sexual/reproductive disorders, nasal congestion, and diarrhea (Wahlestedt et al., 1993; Dumont et al., 1992). The available data implicate this receptor in the control of obesity and other disorders of feeding including bulimia and anorexia. The chemical synthesis of selective drugs not only for Y4 but for all "Y type" receptors will be greatly accelerated by preliminary screening against a homogeneous population of cloned human Y4 receptors. As more specific pharmacological tools become available for probing receptor function, additional therapeutic indications are likely to be discovered.

[0126] Applicants do not know whether hp25a represents the single Y4 receptor expressed in the human genome, or whether there exists a group of structurally related Y4 receptor subtypes. This is an issue which can be resolved using nucleotide sequences from Y4 receptor as the basis for *in situ* localization, antisense or "knockout" strategies, homology cloning, and related techniques. Such approaches will enable one to investigate the existence of potentially novel receptor subtypes with pharmacologic and therapeutic significance.

[0127] In conclusion, the primary structure of the proteins encoded by hp25a (Y4) gene and its homolog in the rat, as well as its unique pharmacological profile obtained for the Y4 receptor subtype, indicate that these genes represent a new pancreatic polypeptide receptor subfamily. Additional cloning efforts will be required to isolate additional members of this newly recognized neuropeptide receptor family.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Bard, Jonathan A.
Walker, Mary
Branchek, Theresa
Weinshank, Richard L.

(ii) TITLE OF INVENTION: DNA ENCODING A HUMAN NEUROPEPTIDE Y/PEPTIDE YY/PANCREATIC POLYPEPTIDE RECEPTOR (Y4) AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 28

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: White, John P.
(B) REGISTRATION NUMBER: 28,678
(C) REFERENCE/DOCKET NUMBER: 44743-A-PCT\JPW\MAT

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1320 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 88..1212

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGTATTGTTT GTCTGTTTGC CTTGTAGGGC GTCATCCCTC AAGTGTATCA CTTAGTTCAA 60

GAGTCCTGGA ATCTTTTCAC ATCCACT ATG AAC ACC TCT CAC CTC CTG GCC 111
Met Asn Thr Ser His Leu Leu Ala

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                                                1               5

TTG CTG CTC CCA AAA TCT CCA CAA GGT GAA AAC AGA AGC AAA CCC CTG      159
Leu Leu Leu Pro Lys Ser Pro Gln Gly Glu Asn Arg Ser Lys Pro Leu
     10                  15                  20

GGC ACC CCA TAC AAC TTC TCT GAA CAT TGC CAG GAT TCC GTG GAC GTG      207
Gly Thr Pro Tyr Asn Phe Ser Glu His Cys Gln Asp Ser Val Asp Val
 25                  30                  35                  40

ATG GTC TTC ATC GTC ACT TCC TAC AGC ATT GAG ACT GTC GTG GGG GTC      255
Met Val Phe Ile Val Thr Ser Tyr Ser Ile Glu Thr Val Val Gly Val
                 45                  50                  55

CTG GGT AAC CTC TGC CTG ATG TGT GTG ACT GTG AGG CAG AAG GAG AAA      303
Leu Gly Asn Leu Cys Leu Met Cys Val Thr Val Arg Gln Lys Glu Lys
             60                  65                  70

GCC AAC GTG ACC AAC CTG CTT ATC GCC AAC CTG GCC TTC TCT GAC TTC      351
Ala Asn Val Thr Asn Leu Leu Ile Ala Asn Leu Ala Phe Ser Asp Phe
         75                  80                  85

CTC ATG TGC CTC CTC TGC CAG CCG CTG ACC GCC GTC TAC ACC ATC ATG      399
Leu Met Cys Leu Leu Cys Gln Pro Leu Thr Ala Val Tyr Thr Ile Met
     90                  95                  100

GAC TAC TGG ATC TTT GGA GAG ACC CTC TGC AAG ATG TCG GCC TTC ATC      447
Asp Tyr Trp Ile Phe Gly Glu Thr Leu Cys Lys Met Ser Ala Phe Ile
105                 110                 115                 120

CAG TGC ATG TCG GTG ACG GTC TCC ATC CTC TCG CTC GTC CTC GTG GCC      495
Gln Cys Met Ser Val Thr Val Ser Ile Leu Ser Leu Val Leu Val Ala
                125                 130                 135

CTG GAG AGG CAT CAG CTC ATC ATC AAC CCA ACA GGC TGG AAG CCC AGC      543
Leu Glu Arg His Gln Leu Ile Ile Asn Pro Thr Gly Trp Lys Pro Ser
            140                 145                 150

ATC TCA CAG GCC TAC CTG GGG ATT GTG CTC ATC TGG GTC ATT GCC TGT      591
Ile Ser Gln Ala Tyr Leu Gly Ile Val Leu Ile Trp Val Ile Ala Cys
        155                 160                 165

GTC CTC TCC CTG CCC TTC CTG GCC AAC AGC ATC CTG GAG AAT GTC TTC      639
Val Leu Ser Leu Pro Phe Leu Ala Asn Ser Ile Leu Glu Asn Val Phe
    170                 175                 180

CAC AAG AAC CAC TCC AAG GCT CTG GAG TTC CTG GCA GAT AAG GTG GTC      687
His Lys Asn His Ser Lys Ala Leu Glu Phe Leu Ala Asp Lys Val Val
185                 190                 195                 200

TGT ACC GAG TCC TGG CCA CTG GCT CAC CAC CGC ACC ATC TAC ACC ACC      735
Cys Thr Glu Ser Trp Pro Leu Ala His His Arg Thr Ile Tyr Thr Thr
                205                 210                 215

TTC CTG CTC CTC TTC CAG TAC TGC CTC CCA CTG GGC TTC ATC CTG GTC      783
Phe Leu Leu Leu Phe Gln Tyr Cys Leu Pro Leu Gly Phe Ile Leu Val
            220                 225                 230

TGT TAT GCA CGC ATC TAC CGG CGC CTG CAG AGG CAG GGG CGC GTG TTT      831
Cys Tyr Ala Arg Ile Tyr Arg Arg Leu Gln Arg Gln Gly Arg Val Phe
        235                 240                 245

CAC AAG GGC ACC TAC AGC TTG CGA GCT GGG CAC ATG AAG CAG GTC AAT      879
His Lys Gly Thr Tyr Ser Leu Arg Ala Gly His Met Lys Gln Val Asn
    250                 255                 260

GTG GTG CTG GTG GTG ATG GTG GTG GCC TTT GCC GTG CTC TGG CTG CCT      927
Val Val Leu Val Val Met Val Val Ala Phe Ala Val Leu Trp Leu Pro
265                 270                 275                 280
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CTG CAT GTG TTC AAC AGC CTG GAA GAC TGG CAC CAT GAG GCC ATC CCC      975
Leu His Val Phe Asn Ser Leu Glu Asp Trp His His Glu Ala Ile Pro
                285                 290                 295

ATC TGC CAC GGG AAC CTC ATC TTC TTA GTG TGC CAC TTG CTT GCC ATG     1023
Ile Cys His Gly Asn Leu Ile Phe Leu Val Cys His Leu Leu Ala Met
            300                 305                 310

GCC TCC ACC TGC GTC AAC CCA TTC ATC TAT GGC TTT CTC AAC ACC AAC     1071
Ala Ser Thr Cys Val Asn Pro Phe Ile Tyr Gly Phe Leu Asn Thr Asn
        315                 320                 325

TTC AAG AAG GAG ATC AAG GCC CTG GTG CTG ACT TGC CAG CAG AGC GCC     1119
Phe Lys Lys Glu Ile Lys Ala Leu Val Leu Thr Cys Gln Gln Ser Ala
    330                 335                 340

CCC CTG GAG GAG TCG GAG CAT CTG CCC CTG TCC ACA GTA CAT ACG GAA     1167
Pro Leu Glu Glu Ser Glu His Leu Pro Leu Ser Thr Val His Thr Glu
345                 350                 355                 360

GTC TCC AAA GGG TCC CTG AGG CTA AGT GGC AGG TCC AAT CCC ATT         1212
Val Ser Lys Gly Ser Leu Arg Leu Ser Gly Arg Ser Asn Pro Ile
                365                 370                 375

TAACCAGGTC TAGGTCTTCT CCCTGCCATG TCCCTTGCCA GGCTCTTCCA CTTAGCTAAG  1272

TGGGCACACT GCAAGCTGGG GTGGCACCCC AGCATTCCTG GCTTTCTG               1320
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 375 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Asn Thr Ser His Leu Leu Ala Leu Leu Leu Pro Lys Ser Pro Gln
  1               5                  10                  15

Gly Glu Asn Arg Ser Lys Pro Leu Gly Thr Pro Tyr Asn Phe Ser Glu
            20                  25                  30

His Cys Gln Asp Ser Val Asp Val Met Val Phe Ile Val Thr Ser Tyr
        35                  40                  45

Ser Ile Glu Thr Val Val Gly Val Leu Gly Asn Leu Cys Leu Met Cys
    50                  55                  60

Val Thr Val Arg Gln Lys Glu Lys Ala Asn Val Thr Asn Leu Leu Ile
65                  70                  75                  80

Ala Asn Leu Ala Phe Ser Asp Phe Leu Met Cys Leu Leu Cys Gln Pro
                85                  90                  95

Leu Thr Ala Val Tyr Thr Ile Met Asp Tyr Trp Ile Phe Gly Glu Thr
            100                 105                 110

Leu Cys Lys Met Ser Ala Phe Ile Gln Cys Met Ser Val Thr Val Ser
        115                 120                 125

Ile Leu Ser Leu Val Leu Val Ala Leu Glu Arg His Gln Leu Ile Ile
    130                 135                 140

Asn Pro Thr Gly Trp Lys Pro Ser Ile Ser Gln Ala Tyr Leu Gly Ile
145                 150                 155                 160
```

```
Val Leu Ile Trp Val Ile Ala Cys Val Leu Ser Leu Pro Phe Leu Ala
                165                 170                 175

Asn Ser Ile Leu Glu Asn Val Phe His Lys Asn His Ser Lys Ala Leu
            180                 185                 190

Glu Phe Leu Ala Asp Lys Val Val Cys Thr Glu Ser Trp Pro Leu Ala
        195                 200                 205

His His Arg Thr Ile Tyr Thr Thr Phe Leu Leu Leu Phe Gln Tyr Cys
    210                 215                 220

Leu Pro Leu Gly Phe Ile Leu Val Cys Tyr Ala Arg Ile Tyr Arg Arg
225                 230                 235                 240

Leu Gln Arg Gln Gly Arg Val Phe His Lys Gly Thr Tyr Ser Leu Arg
                245                 250                 255

Ala Gly His Met Lys Gln Val Asn Val Val Leu Val Val Met Val Val
            260                 265                 270

Ala Phe Ala Val Leu Trp Leu Pro Leu His Val Phe Asn Ser Leu Glu
        275                 280                 285

Asp Trp His His Glu Ala Ile Pro Ile Cys His Gly Asn Leu Ile Phe
    290                 295                 300

Leu Val Cys His Leu Leu Ala Met Ala Ser Thr Cys Val Asn Pro Phe
305                 310                 315                 320

Ile Tyr Gly Phe Leu Asn Thr Asn Phe Lys Lys Glu Ile Lys Ala Leu
                325                 330                 335

Val Leu Thr Cys Gln Gln Ser Ala Pro Leu Glu Glu Ser Glu His Leu
            340                 345                 350

Pro Leu Ser Thr Val His Thr Glu Val Ser Lys Gly Ser Leu Arg Leu
        355                 360                 365

Ser Gly Arg Ser Asn Pro Ile
    370                 375
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTGCTTATGG GGCTGTGATT ATTCTTGGGG TCTCTGGAAA CCTGG

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TAGGATGATT ATGATCAATG CCAGGTTTCC AGAGACCCCA AGAAT 45

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AAAGAGATGA GGAATGTCAC CAACATTCTG ATCGTGAACC TCTCC 45

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAGCAAGTCT GAGAAGGAGA GGTTCACGAT CAGAATGTTG GTGAC 45

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGCAAACTGA ATCCTTTTGT GCAATGCGTC TCCATTACAG TATCCATTTT CTCT 54

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ACGTTCCACA GCGATGAGAA CCAGAGAGAA AATGGATACT GTAATGGAGA CGCA 54

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGCAGTATT TTGGCCCACT CTGTTTCATA TTCATATGCT AC 42

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CAAGCGAATG TATATCTTGA AGTAGCATAT GAATATGAAA CA 42

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTGCTCTGCC ACCTCACGGC CATGATCTCC ACCTGCGTCA ACCCCATC 48

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GAAATTTTTG TTCAGGAATC CATAAAAGAT GGGGTTGACG CAGGTGGA 48

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TCATCGTCAC TTCCTACAGC ATTGAGACTG TCGTGGGGGT CCTGGGT 47

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ACAGTCACAC ACATCAGGCA GAGGTTACCC AGGACCCCCA CGACAG 46

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TGCTTATCGC CAACCTGGCC TTCTCTGACT TCCTCATGTG CCTCC 45

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TAGACGGCGG TCAGCGGCTG GCAGAGGAGG CACATGAGGA AGTCA 45

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TGTCGGCCTT CATCCAGTGC ATGTCGGTGA CGGTCTCCAT CCTCT 45

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTCTCCAGGG CCACGAGGAC GAGCGAGAGG ATGGAGACCG TCACC 45

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCCTACCTGG GGATTGTGCT CATCTGGGTC ATTGCCTGTG TCCTC 45

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TGCTGTTGGC CAGGAAGGGC AGGGAGAGGA CACAGGCAAT GACCC 45

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CATCTACACC ACCTTCCTGC TCCTCTTCCA GTACTGCCTC CCACT 45

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TGCATAACAG ACCAGGATGA AGCCCAGTGG GAGGCAGTAC TGGAA 45

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CTGGTGGTGA TGGTGGTGGC CTTTGCCGTG CTCTGGCTGC CTCTGC 46

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CAGTCTTCCA GGCTGTTGAA CACATGCAGA GGCAGCCAGA GCACG 45

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ATCTTCTTAG TGTGCCACTT GCTTGCCATG GCCTCCACCT GCGTC 45

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TGAGAAAGCC ATAGATGAAT GGGTTGACGC AGGTGGAGGC CATGG 45

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1439 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 178..1306

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATAGCTCTCA AGCCATAAGA TATAAGTAGC TAAGAATTGT CTCCCTCTCC CTGTCCCTTG 60

TTCTTACCTG GTTCCATTTT ACATGCCTGG ACCTTTGAGT TCCATTTGTT TGTTTTGCAG 120

GCTACACTCA GAAGTGGGCC CTTTAGTCTT GAAGTTCCTG GTCTTCTCAC ACCCACC 177

ATG AAT ACC TCT CAT CTC ATG GCC TCC CTT TCT CCG GCA TTC CTA CAA 225
Met Asn Thr Ser His Leu Met Ala Ser Leu Ser Pro Ala Phe Leu Gln
1 5 10 15

GGT AAG AAT GGG ACC AAC CCA CTG GAT TCC CTC TAT AAT CTC TCT GAC 273

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Gly Lys Asn Gly Thr Asn Pro Leu Asp Ser Leu Tyr Asn Leu Ser Asp
                20                  25                  30

GGC TGC CAG GAT TCG GCA GAT CTG TTG GCC TTC ATC ATC ACC ACC TAC      321
Gly Cys Gln Asp Ser Ala Asp Leu Leu Ala Phe Ile Ile Thr Thr Tyr
            35                  40                  45

AGC GTT GAG ACC GTC TTG GGG GTC CTA GGA AAC CTC TGC TTG ATA TTT      369
Ser Val Glu Thr Val Leu Gly Val Leu Gly Asn Leu Cys Leu Ile Phe
        50                  55                  60

GTG ACC ACA AGG CAA AAG GAA AAG TCC AAT GTG ACC AAC CTA CTC ATT      417
Val Thr Thr Arg Gln Lys Glu Lys Ser Asn Val Thr Asn Leu Leu Ile
    65                  70                  75                  80

GCC AAC CTG GCC TTC TCT GAC TTC CTC ATG TGT CTC ATC TGC CAG CCG      465
Ala Asn Leu Ala Phe Ser Asp Phe Leu Met Cys Leu Ile Cys Gln Pro
                85                  90                  95

CTC ACG GTC ACC TAC ACC ATC ATG GAC TAC TGG ATC TTC GGC GAA GTC      513
Leu Thr Val Thr Tyr Thr Ile Met Asp Tyr Trp Ile Phe Gly Glu Val
            100                 105                 110

CTT TGC AAG ATG TTA ACG TTC ATC CAG TGT ATG TCG GTG ACA GTC TCC      561
Leu Cys Lys Met Leu Thr Phe Ile Gln Cys Met Ser Val Thr Val Ser
        115                 120                 125

ATC CTC TCA CTG GTC CTT GTG GCC CTG GAG AGG CAC CAG CTC ATT ATC      609
Ile Leu Ser Leu Val Leu Val Ala Leu Glu Arg His Gln Leu Ile Ile
    130                 135                 140

AAC CCG ACT GGC TGG AAA CCC AGC ATT TCC CAG GCC TAC CTG GGG ATT      657
Asn Pro Thr Gly Trp Lys Pro Ser Ile Ser Gln Ala Tyr Leu Gly Ile
145                 150                 155                 160

GTG GTC ATC TGG TTC ATT TCT TGT TTC CTC TCC TTG CCC TTC CTG GCC      705
Val Val Ile Trp Phe Ile Ser Cys Phe Leu Ser Leu Pro Phe Leu Ala
                165                 170                 175

AAT AGC ATC CTG AAC GAC CTC TTC CAC TAC AAC CAC TCT AAG GTT GTG      753
Asn Ser Ile Leu Asn Asp Leu Phe His Tyr Asn His Ser Lys Val Val
            180                 185                 190

GAG TTT CTG GAA GAC AAG GTT GTC TGC TTT GTG TCC TGG TCC TCG GAT      801
Glu Phe Leu Glu Asp Lys Val Val Cys Phe Val Ser Trp Ser Ser Asp
        195                 200                 205

CAC CAC CGC CTC ATC TAC ACC ACC TTT CTG CTG CTC TTC CAA TAC TGC      849
His His Arg Leu Ile Tyr Thr Thr Phe Leu Leu Leu Phe Gln Tyr Cys
    210                 215                 220

GTC CCT CTG GCC TTC ATC CTG GTC TGC TAC ATG CGT ATC TAT CAG CGC      897
Val Pro Leu Ala Phe Ile Leu Val Cys Tyr Met Arg Ile Tyr Gln Arg
225                 230                 235                 240

CTG CAG AGG CAG AGG CGT GCG TTC CAC ACG CAC ACT TGC AGC TCA CGA      945
Leu Gln Arg Gln Arg Arg Ala Phe His Thr His Thr Cys Ser Ser Arg
                245                 250                 255

GTG GGG CAG ATG AAG CGG ATC AAT GGC ATG CTC ATG GCA ATG GTG ACT      993
Val Gly Gln Met Lys Pro Ile Asn Gly Met Leu Met Ala Met Val Thr
            260                 265                 270

GCC TTT GCA GTT CTC TGG CTG CCC CTG CAT GTG TTC AAC ACT CTG GAG     1041
Ala Phe Ala Val Leu Trp Leu Pro Leu His Val Phe Asn Thr Leu Glu
        275                 280                 285

GAC TGG TAC CAG GAA GCC ATC CCT GCT TGC CAT GGC AAC CTC ATC TTC     1089
Asp Trp Tyr Gln Glu Ala Ile Pro Ala Cys His Gly Asn Leu Ile Phe
    290                 295                 300
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TTG ATG TGC CAC CTG TTT GCC ATG GCT TCC ACC TGT GTC AAC CCT TTC      1137
Leu Met Cys His Leu Phe Ala Met Ala Ser Thr Cys Val Asn Pro Phe
305                 310                 315                 320

ATC TAT GGC TTT CTC AAC ATC AAC TTC AAG AAG GAC ATC AAG GCT CTG      1185
Ile Tyr Gly Phe Leu Asn Ile Asn Phe Lys Lys Asp Ile Lys Ala Leu
                325                 330                 335

GTT CTG ACC TGC CGT TGC AGG CCA CCT CAA GGG GAG CCT GAG CCT CTG      1233
Val Leu Thr Cys Arg Cys Arg Pro Pro Gln Gly Glu Pro Glu Pro Leu
            340                 345                 350

CCC CTG TCC ACT GTG CAC ACG GAC CTC TCC AAG GGA TCT ATG AGG ATG      1281
Pro Leu Ser Thr Val His Thr Asp Leu Ser Lys Gly Ser Met Arg Met
        355                 360                 365

GGT AGC AAG TCT AAC GTC ATG TAG T CATGTCTAGG CTCTTCCGCC              1326
Gly Ser Lys Ser Asn Val Met  *
    370                 375

ATTTCTTTCG ACACACCCTT TCACTGAGCT AAGTAGACAC AATGCAAGCT GTGGTATCAT    1386

CCTGCCATTT CTGGTCTTTG GGGCCCAGAC AGGCGGCAAG AGACTTGAAG CTT           1439
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(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 376 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

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Met Asn Thr Ser His Leu Met Ala Ser Leu Ser Pro Ala Phe Leu Gln
 1               5                  10                  15

Gly Lys Asn Gly Thr Asn Pro Leu Asp Ser Leu Tyr Asn Leu Ser Asp
            20                  25                  30

Gly Cys Gln Asp Ser Ala Asp Leu Leu Ala Phe Ile Ile Thr Thr Tyr
        35                  40                  45

Ser Val Glu Thr Val Leu Gly Val Leu Gly Asn Leu Cys Leu Ile Phe
    50                  55                  60

Val Thr Thr Arg Gln Lys Glu Lys Ser Asn Val Thr Asn Leu Leu Ile
65                  70                  75                  80

Ala Asn Leu Ala Phe Ser Asp Phe Leu Met Cys Leu Ile Cys Gln Pro
                85                  90                  95

Leu Thr Val Thr Tyr Thr Ile Met Asp Tyr Trp Ile Phe Gly Glu Val
            100                 105                 110

Leu Cys Lys Met Leu Thr Phe Ile Gln Cys Met Ser Val Thr Val Ser
        115                 120                 125

Ile Leu Ser Leu Val Leu Val Ala Leu Glu Arg His Gln Leu Ile Ile
    130                 135                 140

Asn Pro Thr Gly Trp Lys Pro Ser Ile Ser Gln Ala Tyr Leu Gly Ile
145                 150                 155                 160

Val Val Ile Trp Phe Ile Ser Cys Phe Leu Ser Leu Pro Phe Leu Ala
                165                 170                 175

Asn Ser Ile Leu Asn Asp Leu Phe His Tyr Asn His Ser Lys Val Val
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                180                 185                 190

Glu Phe Leu Glu Asp Lys Val Val Cys Phe Val Ser Trp Ser Ser Asp
        195                 200                 205

His His Arg Leu Ile Tyr Thr Thr Phe Leu Leu Leu Phe Gln Tyr Cys
    210                 215                 220

Val Pro Leu Ala Phe Ile Leu Val Cys Tyr Met Arg Ile Tyr Gln Arg
225                 230                 235                 240

Leu Gln Arg Gln Arg Arg Ala Phe His Thr His Thr Cys Ser Ser Arg
                245                 250                 255

Val Gly Gln Met Lys Pro Ile Asn Gly Met Leu Met Ala Met Val Thr
            260                 265                 270

Ala Phe Ala Val Leu Trp Leu Pro Leu His Val Phe Asn Thr Leu Glu
        275                 280                 285

Asp Trp Tyr Gln Glu Ala Ile Pro Ala Cys His Gly Asn Leu Ile Phe
    290                 295                 300

Leu Met Cys His Leu Phe Ala Met Ala Ser Thr Cys Val Asn Pro Phe
305                 310                 315                 320

Ile Tyr Gly Phe Leu Asn Ile Asn Phe Lys Lys Asp Ile Lys Ala Leu
                325                 330                 335

Val Leu Thr Cys Arg Cys Arg Pro Pro Gln Gly Glu Pro Glu Pro Leu
            340                 345                 350

Pro Leu Ser Thr Val His Thr Asp Leu Ser Lys Gly Ser Met Arg Met
        355                 360                 365

Gly Ser Lys Ser Asn Val Met  *
    370                 375
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Claims

1. An isolated nucleic acid molecule encoding a human Y4 receptor, i.e., a receptor characterized by a pharmacological profile characteristic of the human Y4 receptor as shown in Table 6.

2. An isolated nucleic acid molecule encoding a rat Y4 receptor, for example a receptor characterized by a pharmacological profile characteristic of the rat Y4 receptor as shown in Table 6.

3. The nucleic acid molecule of claim 1 or 2, wherein the nucleic acid molecule is a DNA molecule or an RNA molecule.

4. The DNA molecule of claim 3, wherein the DNA molecule is a cDNA molecule.

5. The DNA molecule of claim 3, wherein the DNA molecule is a genomic DNA molecule.

6. The nucleic acid molecule of claim 1, wherein the human Y4 receptor has substantially the same amino acid sequence as that shown in Figure 1.

7. The nucleic acid molecule of claim 1, wherein the human Y4 receptor has the amino acid sequence shown in Figure 1.

8. The nucleic acid molecule of claim 2, wherein the rat Y4 receptor has substantially the same amino acid sequence

as that shown in Figure 3.

9. The nucleic acid molecule of claim 8, wherein the rat Y4 receptor has the amino acid sequence shown in Figure 3.

10. A purified Y4 receptor protein encoded by the nucleic acid molecule of any of claims 1 to 5.

11. A vector comprising the nucleic acid molecule of any of claims 1 to 9.

12. A vector of claim 11 adapted for expression in a bacterial cell, yeast cell, insect cell, or mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in said cell operatively linked to the DNA encoding the Y4 receptor as to permit expression thereof.

13. The vector of claim 12, wherein the vector is a baculovirus.

14. The vector of claim 12, wherein the vector is a plasmid.

15. The plasmid of claim 14 designated pcEXV-Y4 (ATCC Accession No. 75631).

16. A mammalian cell comprising the vector of claim 13 or 14.

17. The cell of claim 16, wherein the cell is non-neuronal in origin.

18. The cell of claim 16, wherein the cell is a COS-7 cell or an LM(tk-) cell.

19. The cell of claim 16, wherein the cell is an NIH-3T3 cell.

20. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human Y4 receptor of claim 1 or a rat Y4 receptor of claim 2.

21. The nucleic acid probe of claim 20, wherein the nucleic acid is DNA.

22. The nucleic acid probe of claim 20, wherein the nucleic acid is RNA.

23. An antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human Y4 receptor of claim 1 or a rat Y4 receptor of claim 2 so as to prevent translation of the mRNA molecule or having a sequence capable of specifically hybridizing to the cDNA molecule of claim 4.

24. The antisense oligonucleotide of claim 23 comprising chemical analogues of nucleotides.

25. An antibody capable of binding to a human Y4 receptor encoded by the nucleic acid molecule of claim 1 or a rat Y4 receptor encoded by the nucleic acid molecule of claim 2.

26. An antibody capable of competitively inhibiting the binding of the antibody of claim 25 to a human Y4 receptor encoded by the nucleic acid molecule of claim 1 or a rat Y4 receptor encoded by the nucleic acid molecule of claim 2.

27. The antibody of claim 26, wherein the antibody is a monoclonal antibody.

28. The monoclonal antibody of claim 27 directed to an epitope of a human or rat Y4 receptor present on the surface of a human or rat Y4 receptor expressing cell.

29. A pharmaceutical composition comprising an amount of the oligonucleotide or claim 23 or 24 effective to decrease activity of a human or rat Y4 receptor by passing through a cell membrane and binding specifically with mRNA encoding a human or rat Y4 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

30. The pharmaceutical composition of claim 29, wherein the oligonucleotide is coupled to a substance which inacti-

vates mRNA.

31. The pharmaceutical composition of claim 30, wherein the substance which inactivates mRNA is a ribozyme.

32. The pharmaceutical composition or any of claims 29 to 31, wherein the pharmaceutically acceptable carrier comprises a structure which binds to a receptor on a cell capable of being taken up by cells after binding to the structure.

33. The pharmaceutical composition of claim 32, wherein the structure of the pharmaceutically acceptable carrier is capable of binding to a receptor which is specific for a selected cell type.

34. A pharmaceutical composition which comprises an amount of the antibody of claim 25 effective to block binding of a ligand to a human or rat Y4 receptor and a pharmaceutically acceptable carrier.

35. A method for determining whether a ligand can bind specifically to a human or rat Y4 receptor which comprises contacting a plurality of cells transfected with and expressing nucleic acid encoding the human Y4 receptor of claim 1 or the rat Y4 receptor of claim 2 with the ligand under conditions permitting binding of ligands to such receptor, and detecting the presence of the ligand bound specifically to the Y4 receptor, thereby determining whether the ligand binds specifically to a human or rat Y4 receptor.

36. A method for determining whether a ligand can bind specifically to a human or rat Y4 receptor which comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding the human Y4 receptor of claim 1 or the rat Y4 receptor of claim 2, isolating a membrane fraction from the cell extract, contacting the ligand with the membrane fraction under conditions permitting binding of ligands to such receptor, and detecting the presence of any ligand bound to the Y4 receptor, thereby determining whether the ligand is capable of specifically binding to a human or rat Y4 receptor.

37. A method for determining whether a ligand is a human or rat Y4 receptor agonist which comprises contacting a plurality of cells transfected with and expressing nucleic acid encoding a human Y4 receptor of claim 1 or a rat Y4 receptor of claim 2 with the ligand under conditions permitting the activation of a functional Y4 receptor response, and detecting by means of a bioassay, such as a second messenger assay, an increase in Y4 receptor activity, thereby determining whether the ligand is a human or rat Y4 receptor agonist.

38. A method for determining whether a ligand is a human or rat Y4 receptor agonist which comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding a human Y4 receptor of claim 1 or a rat Y4 receptor of claim 2, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand under conditions permitting the activation of a functional Y4 receptor response and detecting by means of a bioassay, such as a second messenger assay, an increase in Y4 receptor activity, thereby determining whether the ligand is a human or rat Y4 receptor agonist.

39. A method for determining whether a ligand is a human or rat Y4 receptor antagonist which comprises contacting a plurality of cells transfected with and expressing nucleic acid encoding a human Y4 receptor of claim 1 or a rat Y4 receptor of claim 2 with the ligand in the presence of a known human or rat Y4 receptor agonist under conditions permitting the activation of a functional Y4 receptor response and detecting by means of a bioassay, such as a second messenger assay, a decrease in Y4 receptor activity, thereby determining whether the ligand is a human or rat Y4 receptor antagonist.

40. A method for determining whether a ligand is a human or rat Y4 receptor antagonist which comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding a human Y4 receptor of claim 1 or a rat Y4 receptor of claim 2, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand in the presence of a known human or rat Y4 receptor agonist under conditions permitting the activation of a functional Y4 receptor response and detecting by means of a bioassay, such as a second messenger assay, a decrease in Y4 receptor activity, thereby determining whether the ligand is a human or rat Y4 receptor antagonist.

41. The method of any of claims 37 to 40, wherein the second messenger assay comprises measurement of intracellular cAMP or intracellular calcium mobilization.

42. The method of any of claims 37 to 41, wherein the cell is a mammalian cell.

43. The method of claim 42, wherein the mammalian cell is nonneuronal in origin.

44. The method of claim 43, wherein the mammalian cell, of nonneuronal origin is a COS-7 cell, CHO cell, LM(tk-) cell or NIH-3T3 cell.

45. A method of screening chemical compounds to identify drug candidates which specifically bind to a human or rat Y4 receptor on the surface of a cell which comprises contacting a plurality of cells transfected with and expressing nucleic acid encoding a human Y4 receptor of claim 1 or a rat Y4 receptor of claim 2 with a plurality of compounds under conditions permitting binding of compounds to the Y4 receptor, and determining those compounds which specifically bind to the transfected cell, thereby identifying drug candidates which specifically bind to a human or rat Y4 receptor.

46. A method of screening chemical compounds to identify drug candidates which specifically bind to a human or rat Y4 receptor on the surface of a cell which comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding a human Y4 receptor of claim 1 or a rat Y4 receptor of claim 2, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of compounds, and determining those compounds which bind to the transfected cell, thereby identifying drug candidates which specifically bind to a human or rat Y4 receptor.

47. A method of screening chemical compounds to identify drug candidates which act as agonists of a human or rat Y4 receptor which comprises contacting a plurality of cells transfected with and expressing nucleic acid encoding a human Y4 receptor of claim 1 or a rat Y4 receptor of claim 2 with a plurality of compounds under conditions permitting the activation of a functional Y4 receptor response, and determining those compounds which activate such receptor using a bioassay, such as a second messenger assay, thereby identifying drug candidates which act as human or rat Y4 receptor agonists.

48. A method of screening chemical compounds to identify drug candidates which act as agonists of a human or rat Y4 receptor which comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding a human Y4 receptor of claim 1 or a rat Y4 receptor of claim 2, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of compounds under conditions permitting the activation of a functional Y4 receptor response, and determining those compounds which activate such receptor using a bioassay, such as a second messenger assay, thereby identifying drug candidates which act as human or rat Y4 receptor agonists.

49. A method of screening chemical compounds to identify drug candidates which act as a human or rat Y4 receptor antagonists which comprises contacting a plurality of cells transfected with and expressing nucleic acid encoding a human Y4 receptor of claim 1 or a rat Y4 receptor of claim 2, with a plurality of compounds in the presence of a known human or rat Y4 receptor agonist under conditions permitting the activation of a functional Y4 receptor response, and determining those compounds which inhibit the activation of the receptor using a bioassay, such as a second messenger assay, thereby identifying drug candidates which act as human or rat Y4 receptor antagonists.

50. A method of screening chemical compounds to identify drug candidates which act as human or rat Y4 receptor antagonists which comprises contacting a membrane fraction obtained from a cell transfected with and expressing nucleic acid encoding a human Y4 receptor of claim 1 or a rat Y4 receptor of claim 2 with a plurality of compounds in the presence of a known human or rat Y4 receptor agonist under conditions permitting the activation of a functional Y4 receptor response, and determining those compounds which inhibit the activation of the receptor using a bioassay, such as a second messenger assay, thereby identifying drug candidates which act as human or rat Y4 receptor antagonists.

51. The method of any of claims 45 to 50, wherein the second messenger assay comprises measurement of intracellular cAMP or intracellular calcium mobilization.

52. The method of any of claims 45 to 51, wherein the cell is a mammalian cell.

53. The method of claim 52, wherein the mammalian cell is nonneuronal in origin.

54. The method of claim 53 wherein the mammalian cell of nonneuronal origin is a COS-7 cell, a CHO cell, a LM(tk-) cell or an NIH-3T3 cell.

55. A method of detecting expression of a human or rat Y4 receptor by detecting the presence of mRNA coding for such human or rat Y4 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the nucleic acid probe of any of claims 20 to 22 under hybridizing conditions, and detecting the presence of mRNA hybridized to the probe, thereby detecting the expression of a human or rat Y4 receptor by the cell.

56. A method of detecting the presence of a human or rat Y4 receptor on the surface of a cell which comprises contacting the cell with the antibody of claim 25 under conditions permitting binding of the antibody to the receptor, and detecting the presence of the antibody bound to the cell, thereby detecting the presence of a human or rat Y4 receptor on the surface of the cell.

57. A composition comprising the nucleic acid probe of any of claims 20 to 22 for diagnosing a predisposition to a disorder associated with the activity of a specific human Y4 receptor allele by:

a. obtaining DNA of subjects suffering from the disorder;

b. performing a restriction digest of the DNA with a panel of restriction enzymes;

c. electrophoretically separating the resulting DNA fragments on a sizing gel;

d. contacting the resulting gel with said nucleic acid probe capable of specifically hybridizing to DNA encoding a human Y4 receptor and labeled with a detectable marker;

e. detecting labeled bands which have hybridized to the DNA encoding a human Y4 receptor labeled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder;

f. preparing DNA obtained for diagnosis by steps a-e; and

g. comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

58. The composition of claim 57, wherein a disorder associated with the expression of a specific human Y4 receptor allele is diagnosed.

59. A method of preparing the purified human or rat Y4 receptor of claim 11 which comprises:

a. constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of nucleic acid in the cell operatively linked to the nucleic acid encoding the human or rat Y4 receptor as to permit expression thereof, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, insect cells and mammalian cells;

b. inserting the vector of step a in a suitable host cell;

c. incubating the cells of step b under conditions allowing the expression of the human or rat Y4 receptor;

d. recovering the receptor so produced; and

e. purifying the receptor so recovered, thereby preparing a human or rat Y4 receptor.

60. A membrane preparation isolated from a cell which does not normally express a human or rat Y4 receptor but has been transfected with DNA encoding a human Y4 receptor encoded by the nucleic acid molecule according to claim 1 or a rat Y4 receptor according to claim 2, under conditions such that the human or rat Y4 receptor is expressed on the surface of the cells.

61. A process for identifying a chemical compound which specifically binds to a human or rat Y4 receptor, which com-

prises contacting nonneuronal cells expressing on their cell surface the human Y4 receptor encoded by the nucleic acid of claim 1 or the rat Y4 receptor encoded by the nucleic acid of claim 2, or a membrane fraction from a cell extract thereof, with the chemical compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the human or rat Y4 receptor.

62. A process involving competitive binding for identifying a chemical compound which specifically binds to a human or rat Y4 receptor, which comprises separately contacting nonneuronal cells expressing on their cell surface a human Y4 receptor encoded by the nucleic acid of claim 1 or a rat Y4 receptor encoded by the nucleic acid of claim 2, or a membrane fraction from a cell extract thereof, with both the chemical compound and a second chemical compound known to bind to the Y4 receptor, and with only the second chemical compound, under conditions suitable for binding of both chemical compounds, and detecting specific binding of the chemical compound to the Y4 receptor, a decrease in the binding of the second chemical compound to the Y4 receptor in the presence of the chemical compound indicating that the chemical compound binds to the human or Y4 receptor.

63. A process for determining whether a chemical compound specifically binds to and activates a human or rat Y4 receptor, which comprises contacting nonneuronal cells producing a second messenger response and expressing on their cell surface a human Y4 receptor encoded by the nucleic acid of claim 1 or a rat Y4 receptor encoded by the nucleic acid of claim 2, or a membrane fraction from a cell extract thereof, with the chemical compound under conditions suitable for activation of the Y4 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the chemical compound activates the human or rat Y4 receptor.

64. A process for determining whether a chemical compound specifically binds to and inhibits activation of a human or rat Y4 receptor, which comprises separately contacting nonneuronal cells producing a second messenger response and expressing on their cell surface a human Y4 receptor encoded by the nucleic acid of claim 1 or a rat Y4 receptor encoded by the nucleic acid of claim 2, or a membrane fraction from a cell extract thereof, with both the chemical compound and a second chemical compound known to activate a human or rat Y4 receptor, and with only the second chemical compound, under conditions suitable for activation of the Y4 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the human or rat Y4 receptor.

65. The process of claim 63, wherein the second messenger response comprises adenylate cyclase activity and the change in second messenger response is a decrease in adenylate cyclase activity.

66. The process of claim 64, wherein the second messenger response comprises adenylate cyclase activity and the change in second messenger response is a smaller decrease in the level of adenylate cyclase activity in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

67. The process of claim 63, wherein the second messenger response comprises intracellular calcium levels and the change in second messenger response is an increase in intracellular calcium levels.

68. The process of claim 64, wherein the second messenger response comprises intracellular calcium levels and the change in second messenger response is a smaller increase in the level of intracellular calcium in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

69. The process of any of claims 61 to 68, wherein the nonneuronal cell is a mammalian cell.

70. The process of claim 69, wherein the mammalian cell is a COS-7, a CHO cell, an LM(tk-) cell, or an NIH-3T3 cell.

71. Use of a chemical compound for the preparation of a pharmaceutical composition for treating an abnormality, wherein the abnormality is alleviated by decreasing the activity of a human Y4 receptor encoded by a nucleic acid molecule of claim 1.

72. Use of a chemical compound for the preparation of a pharmaceutical composition for treating an abnormality,

wherein the abnormality is alleviated by increasing the activity of a human Y4 receptor encoded by a nucleic acid molecule of claim 1.

73. Use of a compound of claim 71 or 72, wherein the abnormality is amnesia, a feeding disorder, epilepsy, hypertension, sleeping disorder or pain.

74. A method for the production of a pharmaceutical composition comprising the steps of carrying out a screening method according to any one of claims 45 to 50 and mixing the chemical compound identified with the pharmaceutically acceptable carrier.

Patentansprüche

1. Isoliertes Nucleinsäuremolekül, das einen menschlichen Y4-Rezeptor codiert, d. h. einen Rezeptor gekennzeichnet durch ein pharmakologisches Profil, das charakteristisch ist für den menschlichen Y4-Rezeptor wie in Tabelle 6 gezeigt.

2. Isoliertes Nucleinsäuremolekül, das einen Ratten-Y4-Rezeptor codiert, beispielsweise ein Rezeptor gekennzeichnet durch ein pharmakologisches Profil, das charakteristisch ist für den Ratten-Y4-Rezeptor wie in Tabelle 6 gezeigt.

3. Nucleinsäuremolekül nach Anspruch 1 oder 2, wobei das Nucleinsäuremolekül ein DNA-Molekül oder ein RNA-Molekül ist.

4. DNA-Molekül nach Anspruch 3, wobei das DNA-Molekül ein cDNA-Molekül ist.

5. DNA-Molekül nach Anspruch 3, wobei das DNA-Molekül ein genomisches DNA-Molekül ist.

6. Nucleinsäuremolekül nach Anspruch 1, wobei der menschliche Y4-Rezeptor im wesentlichen die gleiche Aminosäuresequenz besitzt, wie die in Figur 1 gezeigte.

7. Nucleinsäuremolekül nach Anspruch 1, wobei der menschliche Y4-Rezeptor die in Figur 1 gezeigte Aminosäuresequenz besitzt.

8. Nucleinsäuremolekül nach Anspruch 2, wobei der Ratten-Y4-Rezeptor im wesentlichen die gleiche Aminosäuresequenz besitzt wie die in Figur 3 gezeigte.

9. Nucleinsäuremolekül nach Anspruch 8, wobei der Ratten-Y4-Rezeptor die in Figur 3 gezeigte Aminosäuresequenz besitzt.

10. Gereinigtes Y4-Rezeptorprotein, das von dem Nucleinsäuremolekül nach einem der Ansprüche 1 bis 5 codiert wird.

11. Vektor, umfassend das Nucleinsäuremolekül nach einem der Ansprüche 1 bis 9.

12. Vektor nach Anspruch 11, der für die Expression in einer bakteriellen Zelle, Hefezelle, Insektenzelle oder Säugerzelle angepaßt ist, umfassend die zur Expression der Nucleinsäure in der Zelle notwendigen regulatorischen Elemente, die wirksam verbunden sind mit der DNA, die den Y4-Rezeptor codiert, um dessen Expression zu erlauben.

13. Vektor nach Anspruch 12, wobei der Vektor ein Baculovirus ist.

14. Vektor nach Anspruch 12, wobei der Vektor ein Plasmid ist.

15. Plasmid nach Anspruch 14 mit der Bezeichnung pcEXV-Y4 (ATCC-Hinterlegungsnummer 75631).

16. Säugerzelle, die den Vektor nach Anspruch 13 oder 14 umfaßt.

17. Zelle nach Anspruch 16, wobei die Zelle nicht-neuronalen Ursprungs ist.

18. Zelle nach Anspruch 16, wobei die Zelle eine COS-7-Zelle oder eine LM(tk-)-Zelle ist.

19. Zelle nach Anspruch 16, wobei die Zelle eine NIH-3T3-Zelle ist.

20. Nucleinsäuresonde, umfassend ein Nucleinsäuremolekül von mindestens 15 Nucleotiden Länge, das in der Lage ist, spezifisch mit einer einzigartigen Sequenz zu hybridisieren, die in der Sequenz des Nucleinsäuremoleküls, das einen menschlichen Y4-Rezeptor nach Anspruch 1 oder einen Ratten-Y4-Rezeptor nach Anspruch 2 codiert, enthalten ist.

21. Nucleinsäuresonde nach Anspruch 20, wobei die Nucleinsäure DNA ist.

22. Nucleinsäuresonde nach Anspruch 20, wobei die Nucleinsäure RNA ist.

23. Antisense-Oligonucleotid, das eine Sequenz besitzt, die in der Lage ist, spezifisch an ein mRNA-Molekül zu hybridisieren, das den menschlichen Y4-Rezeptor nach Anspruch 1 oder den Ratten-Y4-Rezeptor nach Anspruch 2 codiert, um die Translation des mRNA-Moleküls zu verhindern oder eine Sequenz besitzt, die in der Lage ist, spezifisch an das cDNA-Molekül nach Anspruch 4 zu hybridisieren.

24. Antisense-Oligonucleotid nach Anspruch 23, das chemische Analoga von Nucleotiden umfaßt.

25. Antikörper, der in der Lage ist, einen menschlichen Y4-Rezeptor, der von dem Nucleinsäuremolekül nach Anspruch 1 codiert wird, oder einen Ratten-Y4-Rezeptor, der von dem Nucleinsäuremolekül nach Anspruch 2 codiert wird, zu binden.

26. Antikörper, der in der Lage ist, die Bindung des Antikörpers nach Anspruch 25 an einen menschlichen Y4-Rezeptor, der von dem Nucleinsäuremolekül nach Anspruch 1 codiert wird, oder einen Ratten-Y4-Rezeptor, der von dem Nucleinsäuremolekül nach Anspruch 2 codiert wird, kompetitiv zu hemmen.

27. Antikörper nach Anspruch 26, wobei der Antikörper ein monoclonaler Antikörper ist.

28. Monoclonaler Antikörper nach Anspruch 27, der gegen ein Epitop eines menschlichen oder Ratten-Y4-Rezeptors gerichtet ist, das sich an der Oberfläche einer Zelle befindet, die einen menschlichen oder Ratten-Y4-Rezeptor exprimiert.

29. Arzneimittel, das eine Menge des Oligonucleotids nach Anspruch 23 oder 24 umfaßt, die wirksam ist, um die Aktivität eines menschlichen oder Ratten-Y4-Rezeptors zu vermindern, durch Durchdringen der Zellmembran und spezifisches Binden mit mRNA, die einen menschlichen oder Ratten-Y4-Rezeptor in der Zelle codiert, um dessen Translation zu verhindern, und einen pharmazeutisch verträglichen Träger, der in der Lage ist, die Zellmembran zu durchdringen.

30. Arzneimittel nach Anspruch 29, wobei das Oligonucleotid an eine Substanz gebunden ist, die mRNA inaktiviert.

31. Arzneimittel nach Anspruch 30, wobei die Substanz, die mRNA inaktiviert, ein Ribozym ist.

32. Arzneimittel nach einem der Ansprüche 29 bis 31, wobei der pharmazeutisch verträgliche Träger eine Struktur umfaßt, die einen Rezeptor auf einer Zelle bindet, der in der Lage ist, von Zellen aufgenommen zu werden, nach der Bindung an die Struktur.

33. Arzneimittel nach Anspruch 32, wobei die Struktur des phamazeutisch verträglichen Trägers in der Lage ist, einen Rezeptor zu binden, der spezifisch für einen ausgewählten Zelltyp ist.

34. Arzneimittel, das eine Menge des Antikörpers nach Anspruch 25 umfaßt, die wirksam ist, um die Bindung eines Liganden an einen menschlichen oder Ratten-Y4-Rezeptor zu blockieren, und einem pharmazeutisch verträglichen Träger.

35. Verfahren zur Bestimmung ob ein Ligand spezifisch an einen menschlichen oder Ratten-Y4-Rezeptor binden kann, umfassend das Inkontaktbringen einer Vielzahl von Zellen, die mit Nucleinsäure, die den menschlichen Y4-Rezeptor nach Anspruch 1 oder den Ratten-Y4-Rezeptor nach Anspruch 2 codiert, transfiziert sind und diese exprimie-

ren, mit dem Ligand unter Bedingungen, die die Bindung von Liganden an einen solchen Rezeptor erlauben, und Feststellen der Anwesenheit des spezifisch an den Y4-Rezeptor gebundenen Liganden, dadurch Bestimmen, ob der Ligand spezifisch an einen menschlichen oder Ratten-Y4-Rezeptor bindet.

36. Verfahren zur Bestimmung, ob ein Ligand spezifisch an einen menschlichen oder Ratten-Y4-Rezeptor binden kann, umfassend das Herstellen eines Zellextrakts aus Zellen, die mit Nucleinsäure, die den menschlichen Y4-Rezeptor nach Anspruch 1 oder den Ratten-Y4-Rezeptor nach Anspruch 2 codiert, transfiziert sind und diese exprimieren, Isolieren einer Membranfraktion aus dem Zellextrakt, Inkontaktbringen des Liganden mit der Membranfraktion unter Bedingungen, die das Binden von Liganden an einen solchen Rezeptor erlauben, und Feststellen der Anwesenheit eines Liganden, der an den Y4-Rezeptor gebunden ist, dadurch Bestimmen, ob der Ligand in der Lage ist, spezifisch an einen menschlichen oder Ratten-Y4-Rezeptor zu binden.

37. Verfahren zur Bestimmung, ob ein Ligand ein Agonist für einen menschlichen oder Ratten-Y4-Rezeptor ist, umfassend das Inkontaktbringen einer Vielzahl von Zellen, die mit Nucleinsäure, die den menschlichen Y4-Rezeptor nach Anspruch 1 oder den Ratten-Y4-Rezeptor nach Anspruch 2 codiert, transfiziert sind und diese exprimieren, mit dem Liganden unter Bedingungen, die die Aktivierung einer funktionalen Y4-Rezeptor-Antwort erlauben, und Feststellen einer Erhöhung der Y4-Rezeptor-Aktivität durch einen Bio-Test, wie z. B. einen Second-Messenger-Test, dadurch Bestimmen, ob der Ligand ein Agonist für einen menschlichen oder Ratten-Y4-Rezeptor ist.

38. Verfahren zur Bestimmung, ob ein Ligand ein Agonist für einen menschlichen oder Ratten-Y4-Rezeptor ist, umfassend das Herstellen eines Zellextrakts aus Zellen, die mit Nucleinsäure die einen menschlichen Y4-Rezeptor nach Anspruch 1 oder einen Ratten-Y4-Rezeptor nach Anspruch 2 codiert, transfiziert sind und diese exprimieren, Isolieren einer Membranfraktion aus dem Zellextrakt, Inkontaktbringen der Membranfraktion mit dem Ligand unter Bedingungen, die die Aktivierung einer funktionalen Y4-Rezeptor-Antwort erlauben, und Feststellen einer Erhöhung der Y4-Rezeptor-Aktivität durch einen Bio-Test, wie z. B. einen Second-Messenger-Test, dadurch Bestimmen, ob der Ligand ein Agonist für einen menschlichen oder Ratten-Y4-Rezeptor ist.

39. Verfahren zur Bestimmung, ob ein Ligand ein Antagonist für einen menschlichen oder Ratten-Y4-Rezeptor ist, umfassend das Inkontaktbringen einer Vielzahl von Zellen, die mit Nucleinsäure, die einen menschlichen Y4-Rezeptor nach Anspruch 1 oder einen Ratten-Y4-Rezeptor nach Anspruch 2 codiert, transfiziert sind und diese exprimieren, mit dem Liganden in Anwesenheit eines bekannten menschlichen oder Ratten-Y4-Rezeptor-Agonisten unter Bedingungen, die die Aktivierung einer funktionalen Y4-Rezeptor-Antwort erlauben, und Feststellen einer Verminderung der Y4-Rezeptor-Aktivität durch einen Bio-Test, wie z. B. einen Second-Messenger-Test, dadurch Bestimmen, ob der Ligand ein Antagonist für einen menschlichen oder Ratten-Y4-Rezeptor ist.

40. Verfahren zur Bestimmung, ob ein Ligand ein Antagonist für einen menschlichen oder Ratten-Y4-Rezeptor ist, umfassend das Herstellen eines Zellextrakts aus Zellen, die mit Nucleinsäure, die einen menschlichen Y4-Rezeptor nach Anspruch 1 oder einen Ratten-Y4-Rezeptor nach Anspruch 2 codiert, transfiziert sind und diese exprimieren, Isolieren einer Membranfraktion aus dem Zellextrakt, Inkontaktbringen der Membranfraktion mit dem Liganden in Anwesenheit eines bekannten Agonisten für einen menschlichen oder Ratten-Y4-Rezeptor unter Bedingungen, die die Aktivierung einer funktionalen Y4-Rezeptor-Antwort erlauben, und Feststellen einer Verminderung der Y4-Rezeptor-Aktivität durch einen Bio-Test, wie z. B. einen Second-Messenger-Test, dadurch Bestimmen, ob der Ligand ein Antagonist für einen menschlichen oder Ratten-Y4-Rezeptor ist.

41. Verfahren nach einem der Ansprüche 37 bis 40, wobei der Second-Messenger-Test die Messung von intracellulärem cAMP oder intracellulärer Calcium-Mobilisierung umfaßt.

42. Verfahren nach einem der Ansprüche 37 bis 41, wobei die Zelle eine Säugerzelle ist.

43. Verfahren nach Anspruch 42, wobei die Säugerzelle nicht-neuronalen Ursprungs ist.

44. Verfahren nach Anspruch 43, wobei die Säugerzelle von nicht-neuronalem Urspung eine COS-7-Zelle, CHO-Zelle, LM(tk-)-Zelle oder NIH-3T3-Zelle ist.

45. Verfahren zur Durchmusterung chemischer Verbindungen, um Arzneistoffkandidaten zu identifizieren, die spezifisch an einen menschlichen oder Ratten-Y4-Rezeptor an der Oberfläche einer Zelle binden, umfassend das Inkontaktbringen einer Vielzahl von Zellen, die mit Nucleinsäure, die einen menschlichen Y4-Rezeptor nach Anspruch 1 oder einen Ratten-Y4-Rezeptor nach Anspruch 2 codiert, transfiziert sind und diese exprimieren, mit einer Vielzahl

von Verbindungen unter Bedingungen, die das Binden der Verbindungen an den Y4-Rezeptor erlauben, und Bestimmen der Verbindungen, die spezifisch an die transfizierte Zelle binden, dadurch Identifizieren von Arzneistoffkandidaten, die spezifisch an einen menschlichen oder Ratten-Y4-Rezeptor binden.

46. Verfahren zur Durchmusterung von chemischen Verbindungen, um Arzneistoffkandidaten zu identifizieren, die spezifisch an einen menschlichen oder Ratten-Y4-Rezeptor an der Oberfläche einer Zelle binden, umfassend das Herstellen eines Zellextrakts aus Zellen, die mit Nucleinsäure, die einen menschlichen Y4-Rezeptor nach Anspruch 1 oder einen Ratten-Y4-Rezeptor nach Anspruch 2 codiert, transfiziert sind und diese exprimieren, Isolieren einer Membranfraktion aus dem Zellextrakt, Inkontaktbringen der Membranfraktion mit einer Vielzahl von Verbindungen und Bestimmen der Verbindungen, die an die transfizierte Zelle binden, dadurch Identifizieren von Arzneistoffkandidaten, die spezifisch an einen menschlichen oder Ratten-Y4-Rezeptor binden.

47. Verfahren zur Durchmusterung von chemischen Verbindungen, um Arzneistoffkandidaten zu identifizieren, die als Agonisten für einen menschlichen oder Ratten-Y4-Rezeptor wirken, umfassend das Inkontaktbringen einer Vielzahl von Zellen, die mit Nucleinsäure, die einen menschlichen Y4-Rezeptor nach Anspruch 1 oder einen Ratten-Y4-Rezeptor nach Anspruch 2 codiert, transfiziert sind und diese exprimieren, mit einer Vielzahl von Verbindungen unter Bedingungen, die die Aktivierung einer funktionalen Y4-Rezeptor-Antwort erlauben, und Bestimmen der Verbindungen, die einen solchen Rezeptor aktivieren unter Verwendung eines Bio-Tests, wie z. B. eines Second-Messenger-Tests, dadurch Identifizieren von Arzneistoffkandidaten, die als Agonisten für einen menschlichen oder Ratten-Y4-Rezeptor wirken.

48. Verfahren zur Durchmusterung von chemischen Verbindungen, um Arzneistoffkandidaten zu identifizieren, die als Agonisten für einen menschlichen oder Ratten-Y4-Rezeptor wirken, umfassend das Herstellen eines Zellextrakts aus Zellen, die mit Nucleinsäure, die einen menschlichen Y4-Rezeptor nach Anspruch 1 oder einen Ratten-Y4-Rezeptor nach Anspruch 2 codieren, transfiziert sind und diese exprimieren, Isolieren einer Membranfraktion aus dem Zellextrakt, Inkontaktbringen der Membranfraktion mit einer Vielzahl von Verbindungen unter Bedingungen, die die Aktivierung einer funktionalen Y4-Rezeptor-Antwort erlauben, und Bestimmen der Verbindungen, die einen solchen Rezeptor aktivieren, unter Verwendung eines Bio-Tests, wie z. B. eines Second-Messenger-Tests, dadurch Identifizieren von Arzneistoffkandidaten, die als Agonisten für einen menschlichen oder Ratten-Y4-Rezeptor wirken.

49. Verfahren zur Durchmusterung von chemischen Verbindungen, um Arzneistoffkandidaten zu identifizieren, die als Antagonisten für einen menschlichen oder Ratten-Y4-Rezeptor wirken, umfassend das Inkontaktbringen einer Vielzahl von Zellen, die mit Nucleinsäure, die einen menschlichen Y4-Rezeptor nach Anspruch 1 oder einen Ratten-Y4-Rezeptor nach Anspruch 2 codiert, transfiziert sind und diese exprimieren, mit einer Vielzahl von Verbindungen in Anwesenheit eines bekannten Agonisten für einen menschlichen oder Ratten-Y4-Rezeptor unter Bedingungen, die die Aktivierung einer funktionalen Y4-Rezeptor-Antwort erlauben, und Bestimmen der Verbindungen, die die Aktivierung des Rezeptors hemmen, unter Verwendung eines Bio-Tests, wie z. B. eines Second-Messenger-Tests, dadurch Identifizieren von Arzneistoffkandidaten, die als Antagonisten für einen menschlichen oder Ratten-Y4-Rezeptor wirken.

50. Verfahren zur Durchmusterung von chemischen Verbindungen, um Arzneistoffkandidaten zu identifizieren, die als Antagonisten für einen menschlichen oder Ratten-Y4-Rezeptor wirken, umfassend das Inkontaktbringen einer Membranfraktion, die aus Zellen erhalten wurde, die mit Nucleinsäure, die einen menschlichen Y4-Rezeptor nach Anspruch 1 oder einen Ratten-Y4-Rezeptor nach Anspruch 2 codiert, transfiziert sind und diese exprimieren, mit einer Vielzahl von Verbindungen in Anwesenheit eines bekannten Agonisten für einen menschlichen oder Ratten-Y4-Rezeptor unter Bedingungen, die die Aktivierung einer funktionalen Y4-Rezeptor-Antwort erlauben, und Bestimmen der Verbindungen, die die Aktivierung des Rezeptors hemmen, unter Verwendung eines Bio-Tests, wie z. B. eines Second-Messenger-Tests, dadurch Identifizieren von Arzneistoffkandidaten, die als Antagonisten für einen menschlichen oder Ratten-Y4-Rezeptor wirken.

51. Verfahren nach einem der Ansprüche 45 bis 50, wobei der Second-Messenger-Test die Messung von intracellulärem cAMP oder intracellulärer Calcium-Mobilisierung umfaßt.

52. Verfahren nach einem der Ansprüche 45 bis 51, wobei die Zelle eine Säugerzelle ist.

53. Verfahren nach Anspruch 52, wobei die Säugerzelle von nicht-neuronalem Ursprung ist.

54. Verfahren nach Anspruch 53, wobei die Säugerzelle von nicht-neuronalem Ursprung eine COS-7-Zelle, CHO-Zelle, eine LM(tk-)-Zelle oder eine NIH-3T3-Zelle ist.

55. Verfahren zum Nachweis der Expression eines menschlichen oder Ratten-Y4-Rezeptors durch Nachweis der Anwesenheit von mRNA, die einen solchen menschlichen oder Ratten-Y4-Rezeptor codiert, umfassend das Erhalten von Gesamt-mRNA aus der Zelle und Inkontaktbringen der so erhaltenen mRNA mit der Nucleinsäuresonde nach einem der Ansprüche 20 bis 22 unter Hybridisierungsbedingungen, und Nachweisen der Anwesenheit von mRNA, die an die Sonde hybridisiert, dadurch Nachweisen der Expression eines menschlichen oder Ratten-Y4-Rezeptors durch die Zelle.

56. Verfahren zum Nachweis der Anwesenheit eines menschlichen oder Ratten-Y4-Rezeptors auf der Oberfläche einer Zelle, umfassend das Inkontaktbringen der Zelle mit dem Antikörper nach Anspruch 25 unter Bedingungen, die das Binden des Antikörpers an den Rezeptor erlauben, und Nachweisen der Anwesenheit des an die Zelle gebundenen Antikörpers, dadurch Nachweisen der Anwesenheit eines menschlichen oder Ratten-Y4-Rezeptors auf der Oberfläche einer Zelle.

57. Zusammensetzung, die die Nucleinsäuresonde nach einem der Ansprüche 20 bis 22 umfaßt, zur Diagnose einer Veranlagung für eine Erkrankung, die mit der Aktivität eines spezifischen menschlichen Y4-Rezeptor-Allels verbunden ist, durch:

a. Erhalten von DNA von Individuen, die an der Erkrankung leiden;
b. Durchführen einer Restriktionsspaltung der DNA mit verschiedenen Restriktionsenzymen;
c. Elektrophoretisches Auftrennen der entstandenen DNA-Fragmente auf einem Gel zur Größenbestimmung;
d. Inkontaktbringen des sich daraus ergebenden Gels mit der Nucleinsäuresonde, die in der Lage ist, spezifisch an DNA, die einen menschlichen Y4-Rezeptor codiert, zu binden, und mit einem nachweisbaren Marker markiert ist;
e. Nachweisen der markierten Banden, die an die DNA hybridisiert haben, die einen menschlichen Y4-Rezeptor codiert und mit einem nachweisbaren Marker markiert ist, um ein einzigartiges Bandenmuster hervorzubringen, das spezifisch ist für die DNA von Individuen, die an der Erkrankung leiden;
f. Herstellen der DNA, die zur Diagnose nach den Schriften a bis e erhalten wurde; und
g. Vergleichen des einzigartigen Bandenmusters, das spezifisch ist für die DNA von Individuen, die an der Erkrankung leiden, aus Schrift e und der DNA, die zur Diagnose erhalten wurde, aus Schrift f, um zu bestimmen, ob das Muster das gleiche oder unterschiedlich ist, und um dadurch die Veranlagung zur Erkrankung zu diagnostizieren, falls die Muster gleich sind.

58. Zusammensetzung nach Anspruch 57, wobei eine Erkrankung, die mit der Expression eines spezifischen menschlichen Y4-Rezeptor-Allels verbunden ist, diagnostiziert wird.

59. Verfahren zur Herstellung des gereinigten menschlichen oder Ratten-Y4-Rezeptors nach Anspruch 11, das umfaßt:

a. Konstruktion eines Vektors, der geeignet ist zur Expression in einer Zelle, umfassend die regulatorischen Elemente, die notwendig sind zur Expression von Nucleinsäure in der Zelle, die funktionell verbunden sind mit der Nucleinsäure, die den menschlichen oder Ratten-Y4-Rezeptor codiert, um dessen Expression zu erlauben, wobei die Zelle ausgewählt ist aus der Gruppe bestehend aus Bakterienzellen, Hefezellen, Insektenzellen und Säugerzellen;
b. Einführen des Vektors nach Schrift a in eine geeignete Wirtszelle;
c. Inkubieren der Zellen nach Schritt b unter Bedingungen, die die Expression des menschlichen oder Ratten-Y4-Rezeptors erlauben;
d. Gewinnen des so hergestellten Rezeptors; und
e. Reinigen des so gewonnenen Rezeptors, dadurch Präparieren eines menschlichen oder Ratten-Y4-Rezeptors.

60. Membranpräparation, die aus einer Zelle isoliert wurde, die normalerweise keinen menschlichen oder Ratten-Y4-Rezeptor exprimiert, aber mit DNA transfiziert wurde, die einen menschlichen Y4-Rezeptor, der von dem Nucleinsäuremolekül nach Anspruch 1 codiert wird, oder einen Ratten-Y4-Rezeptor nach Anspruch 2 codiert, unter solchen Bedingungen, daß der menschliche oder Ratten-Y4-Rezeptor auf der Oberfläche der Zellen exprimiert wird.

61. Verfahren zur Identifizierung einer chemischen Verbindung, die spezifisch an einen menschlichen oder Ratten-Y4-Rezeptor bindet, umfassend das Inkontaktbringen nicht-neuronaler Zellen, die an ihrer Oberfläche den menschlichen Y4-Rezeptor, der von der Nucleinsäure nach Anspruch 1 codiert wird, oder den Ratten-Y4-Rezeptor, der von der Nucleinsäure nach Anspruch 2 codiert wird, exprimieren, oder einer Membranfraktion aus einem Zellextrakt davon mit der chemischen Verbindung unter Bedingungen, die geeignet für das Binden sind, und Nachweisen der spezifischen Bindung der chemischen Verbindung an den menschlichen oder Ratten-Y4-Rezeptor.

62. Verfahren, das kompetitive Bindung zur Identifizierung einer chemischen Verbindung umfaßt, die spezifisch an einen menschlichen oder Ratten-Y4-Rezeptor bindet, umfassend das getrennte Inkontaktbringen von nichtneuronalen Zellen, die an ihrer Oberfläche einen menschlichen Y4-Rezeptor, der von der Nucleinsäure nach Anspruch 1 codiert wird, oder einen Ratten-Y4-Rezeptor, der von der Nucleinsäure nach Anspruch 2 codiert wird, exprimieren, oder eine Membranfraktion aus einem Zellextrakt davon, sowohl mit der chemischen Verbindung als auch einer zweiten chemischen Verbindung, die bekanntermaßen an den Y4-Rezeptor bindet, und nur mit der zweiten chemischen Verbindung, unter Bedingungen, die geeignet sind zur Bindung der beiden chemischen Verbindungen, und Nachweisen der spezifischen Bindung der chemischen Verbindung an den Y4-Rezeptor, wobei eine Verminderung der Bindung der zweiten chemischen Verbindung an den Y4-Rezeptor in Artwesenheit der chemischen Verbindung anzeigt, daß die chemische Verbindung an den menschlichen oder Y4-Rezeptor bindet.

63. Verfahren zur Bestimmung, ob eine chemische Verbindung spezifisch an einen menschlichen oder Ratten-Y4-Rezeptor bindet und diesen aktiviert, umfassend das Inkontaktbringen nicht-neuronaler Zellen, die eine Second-Messenger-Antwort erzeugen und an ihrer Oberfläche einen menschlichen Y4-Rezeptor, der von der Nucleinsäure nach Anspruch 1 codiert wird, oder einen Ratten-Y4-Rezeptor, der von der Nucleinsäure nach Anspruch 2 codiert wird, exprimieren, oder eine Membranfraktion aus einem Zellextrakt davon, mit der chemischen Verbindung unter Bedingungen, die geeignet sind zur Aktivierung des Y4-Rezeptors, und Messen der Second-Messenger-Antwort in Anwesenheit und in Abwesenheit der chemischen Verbindung, wobei eine Änderung der Second-Messenger-Antwort in Anwesenheit der chemischen Verbindung anzeigt, daß die chemische Verbindung den menschlichen oder Ratten-Y4-Rezeptor aktiviert.

64. Verfahren zur Bestimmung, ob eine chemische Verbindung spezifisch an einen menschlichen oder Ratten-Y4-Rezeptor bindet und dessen Aktivierung hemmt, umfassend das getrennte Inkontaktbringen nicht-neuronaler Zellen, die eine Second-Messenger-Antwort erzeugen und auf ihrer Oberfläche einen menschlichen Y4-Rezeptor, der von der Nucleinsäure nach Anspruch 1 codiert wird, oder einen Ratten-Y4-Rezeptor, der von der Nucleinsäure nach Anspruch 2 codiert wird, exprimieren, oder einer Membranfraktion aus einem Zellextrakt davon, sowohl mit der chemischen Verbindung als auch einer zweiten chemischen Verbindung, die bekanntermaßen einen menschlichen oder Ratten-Y4-Rezeptor aktiviert, und nur mit der zweiten chemischen Verbindung, unter Bedingungen, die geeignet sind zur Aktivierung des Y4-Rezeptors, und Messen der Second-Messenger-Antwort in Anwesenheit von nur der zweiten chemischen Verbindung und in Anwesenheit von sowohl der zweiten chemischen Verbindung als auch der chemischen Verbindung, wobei eine kleinere Änderung der Second-Messenger-Antwort in Anwesenheit von sowohl der chemischen Verbindung als auch der zweiten chemischen Verbindung als in Anwesenheit von nur der zweiten chemischen Verbindung anzeigt, daß die chemische Verbindung die Aktivierung des menschlichen oder des Ratten-Y4-Rezeptors hemmt.

65. Verfahren nach Anspruch 63, wobei die Second-Messenger-Antwort Adenylatcyclase-Aktivität umfaßt und die Änderung der Second-Messenger-Antwort eine Verminderung der Adenylatcyclase-Aktivität ist.

66. Verfahren nach Anspruch 64, wobei die Second-Messenger-Antwort Adenylatcyclase-Aktivität umfaßt und die Änderung der Second-Messenger-Antwort eine kleinere Verminderung der Höhe der Adenylatcyclase-Aktivität in Anwesenheit von sowohl der chemischen Verbindung als auch der zweiten chemischen Verbindung als in Anwesenheit von nur der zweiten chemischen Verbindung ist.

67. Verfahren nach Anspruch 63, wobei die Second-Messenger-Antwort intracelluläre Calciumspiegel umfaßt und die Änderung der Second-Messenger-Antwort eine Erhöhung der intracellulären Calciumspiegel ist.

68. Verfahren nach Anspruch 64, wobei die Second-Messenger-Antwort intracelluläre Calciumspiegel umfaßt und die Änderung der Second-Messenger-Antwort ein kleinerer Anstieg des intracellulären Calciumspiegels in Anwesenheit von sowohl der chemischen Verbindung als auch der zweiten chemischen Verbindung als in Anwesenheit von nur der zweiten chemischen Verbindung ist.

69. Verfahren nach einem der Ansprüche 61 bis 68, wobei die nicht-neuronale Zelle eine Säugerzelle ist.

70. Verfahren nach Anspruch 69, wobei die Säugerzelle eine COS-7, eine COH-Zelle, eine LM(tk-)-Zelle oder eine NIH-3T3-Zelle ist.

71. Verwendung einer chemischen Verbindung zur Herstellung eines Arzneimittels zur Behandlung einer Abnormalität, wobei die Abnormalität durch Verminderung der Aktivität des menschlichen Y4-Rezeptors, der durch ein Nucleinsäuremolekül nach Anspruch 1 codiert wird, gemildert wird.

72. Verwendung einer chemischen Verbindung zur Herstellung eines Arzneimittels zur Behandlung einer Abnormalität, wobei die Abnormalität durch Erhöhung der Aktivität eines menschlichen Y4-Rezeptors, der durch ein Nucleinsäuremolekül nach Anspruch 1 codiert wird, gemildert wird.

73. Verwendung einer Verbindung nach Anspruch 71 oder 72, wobei die Abnormalität Amnesie, eine Ernährungsstörung, Epilepsie, Bluthochdruck, Schlafstörung oder Schmerz ist.

74. Verfahren zur Herstellung eines Arzneimittels, das die Schritte zur Durchführung einer Durchmusterungsmethode nach einem der Ansprüche 45 bis 50 umfaßt und Mischen der identifizierten chemischen Verbindung mit einem pharmazeutisch verträglichen Träger.

Revendications

1. Molécule d'acide nucléique isolée codant pour un récepteur Y4 humain, à savoir, un récepteur caractérisé par un profil pharmacologique caractéristique du récepteur Y4 humain tel que présenté dans le tableau 6.

2. Molécule d'acide nucléique isolée codant pour un récepteur Y4 de rat, par exemple un récepteur caractérisé par un profil pharmacologique caractéristique du récepteur Y4 de rat tel que présenté dans le tableau 6.

3. Molécule d'acide nucléique selon la revendication 1 ou 2, dans laquelle la molécule d'acide nucléique est une molécule d'ADN ou une molécule d'ARN.

4. Molécule d'ADN selon la revendication 3, dans laquelle la molécule d'ADN est une molécule d'ADNc.

5. Molécule d'ADN selon la revendication 3, dans laquelle la molécule d'ADN est une molécule d'ADN génomique.

6. Molécule d'acide nucléique selon la revendication 1, dans laquelle le récepteur Y4 humain a essentiellement la même séquence d'acides aminés que celle présentée dans la figure 1.

7. Molécule d'acide nucléique selon la revendication 1, dans laquelle le récepteur Y4 humain a la séquence d'acides aminés présentée dans la figure 1.

8. Molécule d'acide nucléique selon la revendication 2, dans laquelle le récepteur Y4 de rat a essentiellement la même séquence d'acides aminés que celle présentée dans la figure 3.

9. Molécule d'acide nucléique selon la revendication 8, dans laquelle le récepteur Y4 de rat a la séquence d'acides aminés présentée dans la figure 3.

10. Protéine du récepteur Y4 purifiée codée par la molécule d'acide nucléique selon l'une quelconque des revendications 1 à 5.

11. Vecteur comprenant la molécule d'acide nucléique selon l'une quelconque des revendications 1 à 9.

12. Vecteur selon la revendication 11 adapté pour l'expression dans une cellule bactérienne, une cellule de levure, une cellule d'insecte ou une cellule de mammifère qui comprend les éléments de régulation nécessaires à l'expression de l'acide nucléique dans ladite cellule liés de façon fonctionnelle à l'ADN codant pour le récepteur Y4 de façon à permettre son expression.

13. Vecteur selon la revendication 12, dans laquelle le vecteur est un baculovirus.

14. Vecteur selon la revendication 12, dans laquelle le vecteur est un plasmide.

15. Plasmide selon la revendication 14 désigné pcEXV-Y4 (n° ATCC 75631).

16. Cellule de mammifère comprenant le vecteur selon la revendication 13 ou 14.

17. Cellule selon la revendication 16, dans laquelle la cellule est d'origine non neuronale.

18. Cellule selon la revendication 16, dans laquelle la cellule est une cellule COS-7 ou une cellule LM(tk-).

19. Cellule selon la revendication 16, dans laquelle la cellule est une cellule NIH-3T3.

20. Sonde d'acide nucléique comprenant une molécule d'acide nucléique d'au moins 15 nucléotides capable de s'hybrider de manière spécifique avec une séquence unique incluse dans la séquence d'une molécule d'acide nucléique codant pour un récepteur Y4 humain selon la revendication 1 ou un récepteur Y4 de rat selon la revendication 2.

21. Sonde d'acide nucléique selon la revendication 20, dans laquelle l'acide nucléique est un ADN.

22. Sonde d'acide nucléique selon la revendication 20, dans laquelle l'acide nucléique est un ARN.

23. Oligonucléotide antisens ayant une séquence capable de s'hybrider de manière spécifique à une molécule d'ARNm codant pour un récepteur Y4 humain selon la revendication 1 ou un récepteur Y4 de rat selon la revendication 2 de façon à empêcher une traduction de la molécule d'ARNm ou ayant une séquence capable de s'hybrider de manière spécifique à la molécule d'ADNc selon la revendication 4.

24. Oligonucléotide antisens selon la revendication 23, comprenant des analogues chimiques de nucléotides.

25. Anticorps capable de se lier à un récepteur Y4 humain codé par la molécule d'acide nucléique selon la revendication 1 ou un récepteur Y4 de rat codé par la molécule d'acide nucléique selon la revendication 2.

26. Anticorps capable d'inhiber par compétition la liaison de l'anticorps selon la revendication 25 à un récepteur Y4 humain codé par la molécule d'acide nucléique selon la revendication 1 ou un récepteur Y4 de rat codé par la molécule d'acide nucléique selon la revendication 2.

27. Anticorps selon la revendication 26, dans laquelle l'anticorps est un anticorps monoclonal.

28. Anticorps monoclonal selon la revendication 27 dirigé contre un épitope d'un récepteur Y4 humain ou de rat présent à la surface d'une cellule exprimant un récepteur Y4 humain ou de rat.

29. Composition pharmaceutique comprenant une quantité de l'oligonucléotide selon la revendication 23 ou 24 efficace pour diminuer l'activité d'un récepteur Y4 humain ou de rat en traversant une membrane cellulaire et en se liant spécifiquement avec un ARNm codant pour un récepteur Y4 humain ou de rat dans la cellule de façon à empêcher sa traduction et un véhicule acceptable sur le plan pharmaceutique capable de traverser une membrane cellulaire.

30. Composition pharmaceutique selon la revendication 29, dans laquelle l'oligonucléotide est couplé à une substance qui inactive un ARNm.

31. Composition pharmaceutique selon la revendication 30, dans laquelle la substance qui inactive un ARNm est une ribozyme.

32. Composition pharmaceutique selon l'une quelconque des revendications 29 à 31, dans laquelle le véhicule acceptable sur le plan pharmaceutique comprend une structure qui se lie à un récepteur sur une cellule capable d'être absorbé par des cellules après liaison à la structure.

33. Composition pharmaceutique selon la revendication 32, dans laquelle la structure du véhicule acceptable sur le plan pharmaceutique est capable de se lier à un récepteur qui est spécifique d'un type cellulaire choisi.

34. Composition pharmaceutique qui comprend une quantité de l'anticorps selon la revendication 25 efficace pour bloquer une liaison d'un ligand à un récepteur Y4 humain ou de rat et un véhicule acceptable sur le plan pharmaceutique.

35. Procédé pour déterminer si un ligand peut se lier spécifiquement à un récepteur Y4 humain ou de rat qui comprend la mise en contact d'une pluralité de cellules transfectées avec et exprimant un acide nucléique codant pour le récepteur Y4 humain selon la revendication 1 ou le récepteur Y4 de rat selon la revendication 2, avec le ligand dans des conditions permettant la liaison de ligand à un tel récepteur, et la détection de la présence du ligand lié de façon spécifique au récepteur Y4, déterminant ainsi si le ligand se lie de manière spécifique à un récepteur Y4 humain ou de rat.

36. Procédé pour déterminer si un ligand peut se lier spécifiquement à un récepteur Y4 humain ou de rat qui comprend la préparation d'un extrait cellulaire à partir de cellules transfectées avec et exprimant un acide nucléique codant pour le récepteur Y4 humain selon la revendication 1 ou le récepteur Y4 de rat selon la revendication 2, l'isolation d'une fraction membranaire à partir de l'extrait cellulaire, la mise en contact du ligand avec la fraction membranaire dans des conditions permettant la liaison de ligand à un tel récepteur, et la détection de la présence de tout ligand lié au récepteur Y4, déterminant ainsi si le ligand est capable de se lier spécifiquement à un récepteur Y4 humain ou de rat.

37. Procédé pour déterminer si un ligand est un agoniste du récepteur Y4 humain ou de rat qui comprend la mise en contact d'une pluralité de cellules transfectées avec et exprimant un acide nucléique codant pour un récepteur Y4 humain selon la revendication 1 ou un récepteur Y4 de rat selon la revendication 2 avec le ligand dans des conditions permettant l'activation d'une réponse fonctionnelle du récepteur Y4, et la détection au moyen d'un biodosage, tel qu'un dosage de messager secondaire, une augmentation de l'activité du récepteur Y4, déterminant ainsi si le ligand est un agoniste du récepteur Y4 humain ou de rat.

38. Procédé pour déterminer si un ligand est un agoniste du récepteur Y4 humain ou de rat qui comprend la préparation d'un extrait cellulaire à partir de cellules transfectées avec et exprimant un acide nucléique codant pour un récepteur Y4 humain selon la revendication 1 ou un récepteur Y4 de rat selon la revendication 2, l'isolation d'une fraction membranaire à partir de l'extrait cellulaire, la mise en contact de la fraction membranaire avec le ligand dans des conditions permettant l'activation d'une réponse fonctionnelle du récepteur Y4 et la détection au moyen d'un biodosage, tel qu'un dosage de messager secondaire, d'une augmentation de l'activité du récepteur Y4, déterminant ainsi si le ligand est un agoniste du récepteur Y4 humain ou de rat.

39. Procédé pour déterminer si un ligand est un antagoniste du récepteur Y4 humain ou de rat qui comprend la mise en contact d'une pluralité de cellules transfectées avec et exprimant un acide nucléique codant pour un récepteur Y4 humain selon la revendication 1 ou un récepteur Y4 de rat selon la revendication 2 avec le ligand en présence d'un agoniste du récepteur Y4 humain ou de rat connu, dans des conditions permettant l'activation d'une réponse fonctionnelle du récepteur Y4, et la détection au moyen d'un biodosage, tel qu'un dosage de messager secondaire, d'une diminution de l'activité du récepteur Y4, déterminant ainsi si le ligand est un antagoniste du récepteur Y4 humain ou de rat.

40. Procédé pour déterminer si un ligand est un antagoniste du récepteur Y4 humain ou de rat qui comprend la préparation d'un extrait cellulaire à partir de cellules transfectées avec et exprimant un acide nucléique codant pour un récepteur Y4 humain selon la revendication 1 ou un récepteur Y4 de rat selon la revendication 2, l'isolation d'une fraction membranaire à partir de l'extrait cellulaire, la mise en contact de la fraction membranaire avec le ligand en présence d'un agoniste du récepteur Y4 humain ou de rat connu, dans des conditions permettant l'activation d'une réponse fonctionnelle du récepteur Y4, et la détection au moyen d'un biodosage, tel qu'un dosage de messager secondaire, d'une diminution de l'activité du récepteur Y4, déterminant ainsi si le ligand est un antagoniste du récepteur Y4 humain ou de rat.

41. Procédé selon l'une quelconque des revendications 37 à 40, dans lequel le dosage de messager secondaire comprend la mesure de la mobilisation de l'AMPc intracellulaire ou du calcium intracellulaire.

42. Procédé selon l'une quelconque des revendications 37 à 41, dans lequel la cellule est une cellule de mammifère.

43. Procédé selon la revendication 42, dans lequel la cellule de mammifère est d'origine non neuronale.

44. Procédé selon la revendication 43, dans lequel la cellule de mammifère, d'origine non neuronale, est une cellule COS-7, une cellule CHO, une cellule LM(tk-) ou une cellule NIH-3T3.

45. Procédé de criblage de composés chimiques afin d'identifier des candidats médicaments qui se lient spécifiquement à un récepteur Y4 humain ou de rat à la surface d'une cellule qui comprend la mise en contact d'une pluralité de cellules transfectées avec et exprimant un acide nucléique codant pour un récepteur Y4 humain selon la revendication 1 ou un récepteur Y4 de rat selon la revendication 2 avec une pluralité de composés dans des conditions permettant la liaison des composés au récepteur Y4, et la détermination des composés qui se lient spécifiquement à la cellule transfectée, identifiant ainsi les candidats médicaments qui se lient spécifiquement à un récepteur Y4 humain ou de rat.

46. Procédé de criblage de composés chimiques afin d'identifier des candidats médicaments qui se lient spécifiquement à un récepteur Y4 humain ou de rat à la surface d'une cellule qui comprend la préparation d'un extrait cellulaire à partir de cellules transfectées avec et exprimant un acide nucléique codant pour un récepteur Y4 humain selon la revendication 1 ou un récepteur Y4 de rat selon la revendication 2, l'isolation d'une fraction membranaire à partir de l'extrait cellulaire, la mise en contact de l'extrait cellulaire avec une pluralité de composés, et la détermination des composés qui se lient à la cellule transfectée, identifiant ainsi les candidats médicaments qui se lient spécifiquement à un récepteur Y4 humain ou de rat.

47. Procédé de criblage de composés chimiques afin d'identifier des candidats médicaments qui agissent en tant qu'agonistes d'un récepteur Y4 humain ou de rat qui comprend la mise en contact d'une pluralité de cellules transfectées avec et exprimant un acide nucléique codant pour un récepteur Y4 humain selon la revendication 1 ou un récepteur Y4 de rat selon la revendication 2, avec une pluralité de composés dans des conditions permettant l'activation d'une réponse fonctionnelle du récepteur Y4, et la détermination des composés qui activent un tel récepteur en utilisant un biodosage, tel qu'un dosage de messager secondaire, identifiant ainsi les candidats médicaments qui agissent en tant qu'agonistes du récepteur Y4 humain ou de rat.

48. Procédé de criblage de composés chimiques afin d'identifier des candidats médicaments qui agissent en tant qu'agonistes d'un récepteur Y4 humain ou de rat, qui comprend la préparation d'un extrait cellulaire à partir de cellules transfectées avec et exprimant un acide nucléique codant pour un récepteur Y4 humain selon la revendication 1 ou un récepteur Y4 de rat selon la revendication 2, l'isolation d'une fraction membranaire à partir de l'extrait cellulaire, la mise en contact de la fraction membranaire avec une pluralité de composés dans des conditions permettant l'activation d'une réponse fonctionnelle du récepteur Y4, et la détermination des composés qui activent un tel récepteur en utilisant un biodosage, tel qu'un dosage de messager secondaire, identifiant ainsi les candidats médicaments qui agissent en tant qu'agonistes du récepteur Y4 humain ou de rat.

49. Procédé de criblage de composés chimiques afin d'identifier des candidats médicaments qui agissent en tant qu'antagonistes du récepteur Y4 humain ou de rat qui comprend la mise en contact d'une pluralité de cellules transfectées avec et exprimant un acide nucléique codant pour un récepteur Y4 humain selon la revendication 1 ou un récepteur Y4 de rat selon la revendication 2, avec une pluralité de composés en présence d'un agoniste du récepteur Y4 humain ou de rat connu, dans des conditions permettant l'activation d'une réponse fonctionnelle du récepteur Y4, et la détermination des composés qui inhibent l'activation du récepteur en utilisant un biodosage, tel qu'un dosage de messager secondaire, identifiant ainsi les candidats médicaments qui agissent en tant qu'antagonistes du récepteur Y4 humain ou de rat.

50. Procédé de criblage de composés chimiques afin d'identifier des candidats médicaments qui agissent en tant qu'antagonistes du récepteur Y4 humain ou de rat qui comprend la mise en contact d'une fraction membranaire obtenue à partir d'une cellule transfectée avec et exprimant un acide nucléique codant pour un récepteur Y4 humain selon la revendication 1 ou un récepteur Y4 de rat selon la revendication 2 avec une pluralité de composés en présence d'un agoniste du récepteur Y4 humain ou de rat connu, dans des conditions permettant l'activation d'une réponse fonctionnelle du récepteur Y4, et la détermination des composés qui inhibent l'activation du récepteur en utilisant un biodosage, tel qu'un dosage de messager secondaire, identifiant ainsi les candidats médicaments qui agissent en tant qu'antagonistes du récepteur Y4 humain ou de rat.

51. Procédé selon l'une quelconque des revendications 45 à 50, dans lequel le dosage de messager secondaire comprend la mesure de la mobilisation de l'AMPc intracellulaire ou du calcium intracellulaire.

52. Procédé selon l'une quelconque des revendications 45 à 51, dans lequel la cellule est une cellule de mammifère.

53. Procédé selon la revendication 52, dans lequel la cellule de mammifère est d'origine non neuronale.

54. Procédé selon la revendication 53, dans lequel la cellule de mammifère d'origine non neuronale est une cellule COS-7, une cellule CHO, une cellule LM(tk-) ou une cellule NIH-3T3.

55. Procédé de détection de l'expression d'un récepteur Y4 humain ou de rat consistant à détecter la présence d'ARNm codant pour un tel récepteur Y4 humain ou de rat qui comprend l'obtention d'ARNm total provenant de la cellule et la mise en contact de l'ARNm ainsi obtenu avec la sonde d'acide nucléique selon l'une quelconque des revendications 20 à 22 dans des conditions d'hybridisation, et la détection de la présence d'ARNm hybridé à la sonde, détectant ainsi l'expression d'un récepteur Y4 humain ou de rat par la cellule.

56. Procédé de détection de la présence d'un récepteur Y4 humain ou de rat à la surface d'une cellule qui comprend la mise en contact de la cellule avec l'anticorps selon la revendication 25 dans des conditions permettant la liaison de l'anticorps au récepteur, et la détection de la présence de l'anticorps lié à la cellule, détectant ainsi la présence d'un récepteur Y4 humain ou de rat à la surface de la cellule.

57. Composition comprenant la sonde d'acide nucléique selon l'une quelconque des revendications 20 à 22 pour diagnostiquer une prédisposition à un trouble associé à l'activité d'un allèle spécifique du récepteur Y4 humain consistant à :

a. obtenir un ADN de sujets souffrant du trouble ;
b. réaliser une digestion de restriction de l'ADN avec un ensemble d'enzymes de restriction ;
c. séparer par électrophorèse les fragments d'ADN résultants sur un gel de détermination de taille ;
d. mettre en contact le gel résultant avec ladite sonde d'acide nucléique capable de s'hybrider spécifiquement à l'ADN codant pour un récepteur Y4 humain et marquée avec un marqueur détectable ;
e. détecter les bandes marquées qui sont hybridées à l'ADN codant pour un récepteur Y4 humain marqué avec un marqueur détectable afin de créer un motif de bandes unique spécifique à l'ADN des sujets souffrant du trouble ;
f. préparer l'ADN obtenu pour le diagnostic à l'aide des étapes a à e ; et
g. comparer le motif de bandes unique spécifique à l'ADN des sujets souffrant du trouble provenant de l'étape e et l'ADN obtenu pour le diagnostic provenant de l'étape f afin de déterminer si les motifs sont identiques ou différents et pour diagnostiquer ainsi une prédisposition au trouble si les motifs sont identiques.

58. Composition selon la revendication 57, dans laquelle un trouble associé avec l'expression d'un allèle spécifique du récepteur Y4 humain est diagnostiqué.

59. Procédé de préparation du récepteur Y4 humain ou de rat purifié selon la revendication 11 qui comprend :

a. la construction d'un vecteur adapté pour l'expression dans une cellule qui comprend les éléments régulateurs nécessaires pour l'expression d'un acide nucléique dans la cellule liés de façon fonctionnelle à l'acide nucléique codant pour le récepteur Y4 humain ou de rat afin de permettre son expression, la cellule étant choisie dans le groupe constitué des cellules bactériennes, des cellules de levure, des cellules d'insecte et des cellules de mammifère ;
b. l'insertion du vecteur de l'étape a dans une cellule hôte appropriée ;
c. l'incubation des cellules de l'étape b dans des conditions permettant l'expression du récepteur Y4 humain ou de rat ;
d. la récupération du récepteur ainsi produit ; et
e. la purification du récepteur ainsi produit, préparant ainsi un récepteur Y4 humain ou de rat.

60. Préparation membranaire isolée à partir d'une cellule qui n'exprime pas normalement un récepteur Y4 humain ou de rat mais qui a été transfectée avec un ADN codant pour un récepteur Y4 humain codé par la molécule d'acide nucléique selon la revendication 1 ou un récepteur Y4 de rat selon la revendication 2, dans des conditions telles que le récepteur Y4 humain ou de rat est exprimé à la surface des cellules.

61. Procédé pour identifier un composé chimique qui se lie spécifiquement à un récepteur Y4 humain ou de rat, qui comprend la mise en contact de cellules non neuronales exprimant à leur surface cellulaire le récepteur Y4 humain codé par l'acide nucléique selon la revendication 1 ou le récepteur Y4 de rat codé par l'acide nucléique selon la revendication 2, ou d'une fraction membranaire provenant d'un extrait cellulaire de ces cellules, avec le composé

chimique dans des conditions appropriées pour une liaison, et la détection de la liaison spécifique du composé chimique au récepteur Y4 humain ou de rat.

62. Procédé impliquant une compétition de liaison afin d'identifier un composé chimique qui se lie de façon spécifique à un récepteur Y4 humain ou de rat, qui comprend la mise en contact, séparément, de cellules non neuronales exprimant à leur surface cellulaire un récepteur Y4 humain codé par l'acide nucléique selon la revendication 1 ou un récepteur Y4 de rat codé par l'acide nucléique selon la revendication 2, ou d'une fraction membranaire provenant d'un extrait cellulaire de celles-ci, avec à la fois le composé chimique et un second composé chimique connu pour se lier au récepteur Y4, et avec seulement le second composé chimique, dans des conditions appropriées pour une liaison des deux composés chimiques et la détection d'une liaison spécifique du composé chimique au récepteur Y4, une diminution dans la liaison du second composé chimique au récepteur Y4 en présence du composé chimique indiquant que le composé chimique se lie au récepteur Y4 humain ou de rat.

63. Procédé pour déterminer si un composé chimique se lie de façon spécifique à et active un récepteur Y4 humain ou de rat, qui comprend la mise en contact de cellules non neuronales produisant une réponse de messager secondaire et exprimant à leur surface cellulaire un récepteur Y4 humain codé par l'acide nucléique selon la revendication 1 ou un récepteur Y4 de rat codé par l'acide nucléique selon la revendication 2, ou d'une fraction membranaire provenant d'un extrait cellulaire de celles-ci, avec le composé chimique dans des conditions appropriées pour l'activation du récepteur Y4, et la mesure de la réponse de messager secondaire en présence et en l'absence du composé chimique, un changement dans la réponse de messager secondaire en présence du composé chimique indiquant que le composé chimique active le récepteur Y4 humain ou de rat.

64. Procédé pour déterminer si un composé chimique se lie de façon spécifique à et inhibe l'activation d'un récepteur Y4 humain ou de rat, qui comprend la mise en contact, séparément, de cellules non neuronales produisant une réponse de messager secondaire et exprimant à leur surface cellulaire un récepteur Y4 humain codé par l'acide nucléique selon la revendication 1 ou un récepteur Y4 de rat codé par l'acide nucléique selon la revendication 2, ou d'une fraction membranaire provenant d'un extrait cellulaire de celles-ci, avec à la fois le composé chimique et un deuxième composé chimique connu pour activer un récepteur Y4 humain ou de rat, et avec seulement le deuxième composé chimique, dans des conditions appropriées pour l'activation du récepteur Y4, et la mesure de la réponse de messager secondaire en présence seulement du deuxième composé chimique et en présence à la fois du deuxième composé chimique et du composé chimique, un changement plus petit dans la réponse de messager secondaire en présence à la fois du composé chimique et du deuxième composé chimique qu'en la seule présence du deuxième composé chimique indiquant que le composé chimique inhibe l'activation du récepteur Y4 humain ou de rat.

65. Procédé selon la revendication 63, dans lequel la réponse de messager secondaire comprend l'activité de l'adénylate cyclase et le changement de la réponse de messager secondaire est une diminution de l'activité de l'adénylate cyclase.

66. Procédé selon la revendication 64, dans lequel la réponse de messager secondaire comprend l'activité de l'adénylate cyclase et le changement de la réponse de messager secondaire est une diminution plus petite de la valeur de l'activité de l'adénylate cyclase en présence à la fois du composé chimique et du deuxième composé chimique qu'en la seule présence du deuxième composé chimique.

67. Procédé selon la revendication 63, dans lequel la réponse de messager secondaire comprend les niveaux de calcium intracellulaire et le changement de la réponse de messager secondaire est une augmentation des niveaux de calcium intracellulaire.

68. Procédé selon la revendication 64, dans lequel la réponse de messager secondaire comprend les niveaux de calcium intracellulaire et le changement de réponse de messager secondaire est une augmentation plus petite du niveau de calcium intracellulaire en présence à la fois du composé chimique et du deuxième composé chimique qu'en la seule présence du deuxième composé chimique.

69. Procédé selon l'une quelconque des revendications 61 à 68, dans lequel la cellule non neuronale est une cellule de mammifère.

70. Procédé selon la revendication 69, dans lequel la cellule de mammifère est une cellule COS-7, une cellule CHO, une cellule LM(tk-) ou une cellule NIH-3T3.

71. Utilisation d'un composé chimique pour la préparation d'une composition pharmaceutique en vue de traiter une anomalie, dans laquelle l'anomalie est atténuée en diminuant l'activité d'un récepteur Y4 humain codé par une molécule d'acide nucléique selon la revendication 1.

72. Utilisation d'un composé chimique pour la préparation d'une composition pharmaceutique en vue de traiter une anomalie, dans laquelle l'anomalie est atténuée en augmentant l'activité d'un récepteur Y4 humain codé par une molécule d'acide nucléique selon la revendication 1.

73. Utilisation d'un composé selon la revendication 71 ou 72, dans laquelle l'anomalie est une amnésie, un trouble d'alimentation, une épilepsie, une hypertension, un trouble du sommeil ou une douleur.

74. Procédé pour la production d'une composition pharmaceutique comprenant les étapes consistant à réaliser un procédé de criblage selon l'une quelconque des revendications 45 à 50 et à mélanger le composé chimique identifié avec le véhicule acceptable sur le plan pharmaceutique.

FIGURE 1-1


```
          -80                  -60                  -40
           .                    .                    .                    .
-28  AGTATTGTTTGTCTGTTTGCCTTGTAGGGCGTCATCCCTCAAGTGTATCACTTAGTTCAA    31

          -20                  -1                   20
           .                    .                    .                    .
 32  GAGTCCTGGAATCTTTTCACATCCACTATGAACACCTCTCACCTCCTGGCCTTGCTGCTC    91
 -8                                 M  N  T  S  H  L  L  A  L  L  L     11

          40                   60                   80
           .                    .                    .                    .
 92  CCAAAATCTCCACAAGGTGAAAACAGAAGCAAACCCCTGGGCACCCCATACAACTTCTCT    151
 12  P  K  S  P  Q  G  E  N  R  S  K  P  L  G  T  P  Y  N  F  S     31

          100                  120                  140
           .                    .                    .                    .
152  GAACATTGCCAGGATTCCGTGGACGTGATGGTCTTCATCGTCACTTCCTACAGCATTGAG    211
 32  E  H  C  Q  D  S  V  D  V  M  V  F  I  V  T  S  Y  S  I  E     51

          160                  180                  200
           .                    .                    .                    .
212  ACTGTCGTGGGGGTCCTGGGTAACCTCTGCCTGATGTGTGTGACTGTGAGGCAGAAGGAG    271
 52  T  V  V  G  V  L  G  N  L  C  L  M  C  V  T  V  R  Q  K  E     71
```

FIGURE 1-2

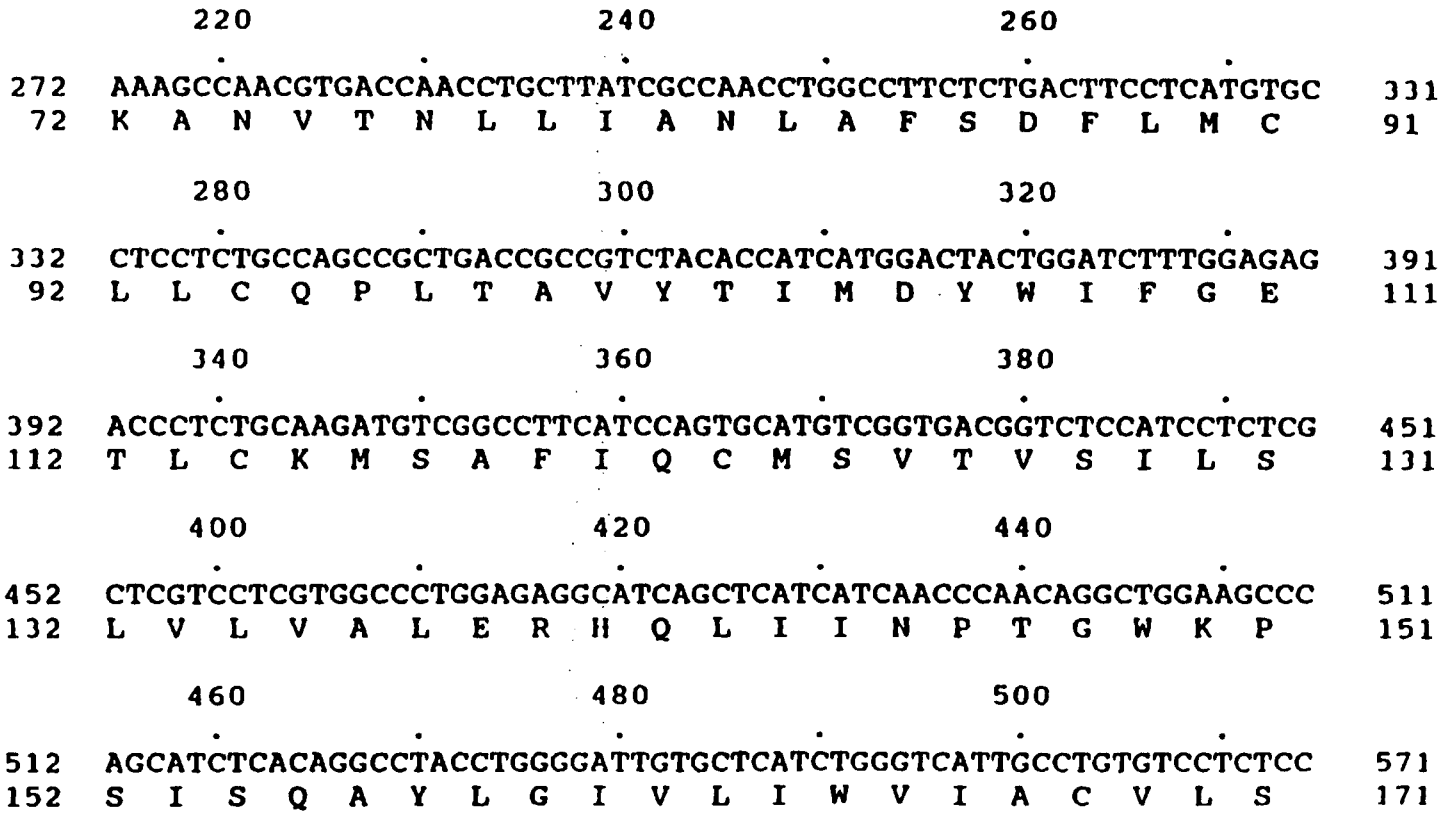

220
240
260
272 AAAGCCAACGTGACCAACCTGCTTATCGCCAACCTGGCCTTCTCTGACTTCCTCATGTGC 331
72 K A N V T N L L I A N L A F S D F L M C 91
280
300
320
332 CTCCTCTGCCAGCCGCTGACCGCCGTCTACACCATCATGGACTACTGGATCTTTGGAGAG 391
92 L L C Q P L T A V Y T I M D Y W I F G E 111
340
360
380
392 ACCCTCTGCAAGATGTCGGCCTTCATCCAGTGCATGTCGGTGACGGTCTCCATCCTCTCG 451
112 T L C K M S A F I Q C M S V T V S I L S 131
400
420
440
452 CTCGTCCTCGTGGCCCTGGAGAGGCATCAGCTCATCATCAACCCAACAGGCTGGAAGCCC 511
132 L V L V A L E R H Q L I I N P T G W K P 151
460
480
500
512 AGCATCTCACAGGCCTACCTGGGGATTGTGCTCATCTGGGTCATTGCCTGTGTCCTCTCC 571
152 S I S Q A Y L G I V L I W V I A C V L S 171

FIGURE 1-3

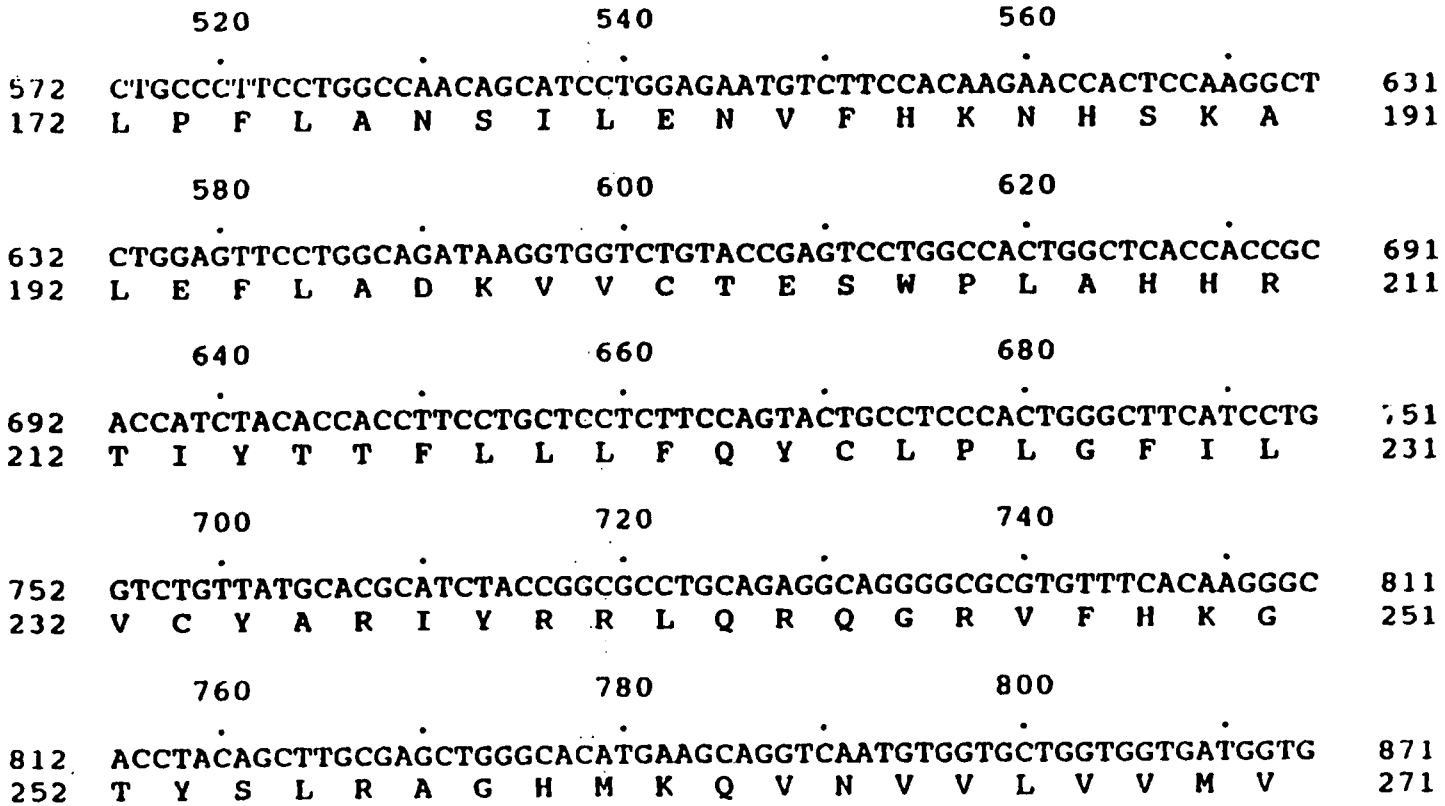
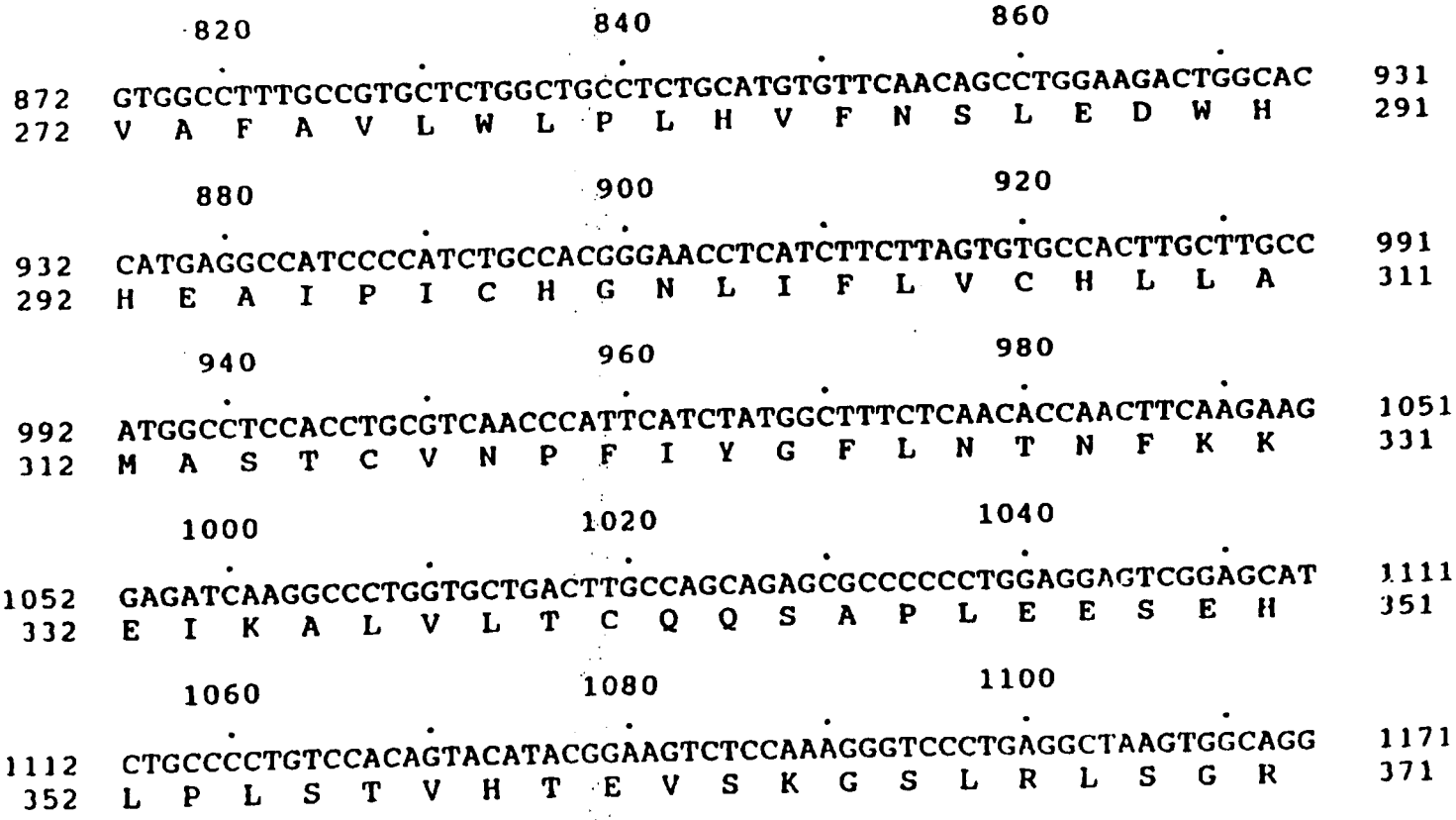

520 540 560
572 CTGCCCTTCCTGGCCAACAGCATCCTGGAGAATGTCTTCCACAAGAACCACTCCAAGGCT 631
172 L P F L A N S I L E N V F H K N H S K A 191
580 600 620
632 CTGGAGTTCCTGGCAGATAAGGTGGTCTGTACCGAGTCCTGGCCACTGGCTCACCACCGC 691
192 L E F L A D K V V C T E S W P L A H H R 211
640 660 680
692 ACCATCTACACCACCTTCCTGCTCCTCTTCCAGTACTGCCTCCCACTGGGCTTCATCCTG 751
212 T I Y T T F L L L F Q Y C L P L G F I L 231
700 720 740
752 GTCTGTTATGCACGCATCTACCGGCGCCTGCAGAGGCAGGGGCGCGTGTTTCACAAGGGC 811
232 V C Y A R I Y R R L Q R Q G R V F H K G 251
760 780 800
812 ACCTACAGCTTGCGAGCTGGGCACATGAAGCAGGTCAATGTGGTGCTGGTGGTGATGGTG 871
252 T Y S L R A G H M K Q V N V V L V V M V 271

FIGURE 1-4



EP 0 746 332 B1

FIGURE 1-5

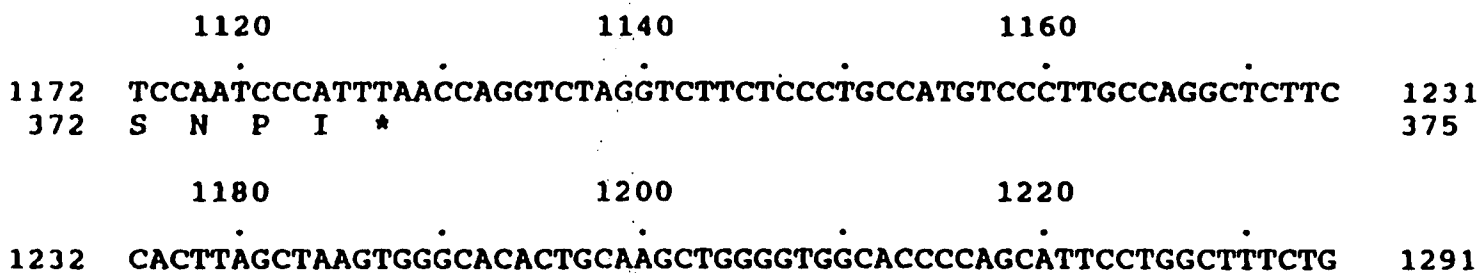

1120
1140
1160
1172 TCCAATCCCATTTAACCAGGTCTAGGTCTTCTCCCTGCCATGTCCCTTGCCAGGCTCTTC 1231
372 S N P I *
375
1180
1200
1220
1232 CACTTAGCTAAGTGGGCACACTGCAAGCTGGGGTGGCACCCCAGCATTCCTGGCTTTCTG 1291

FIGURE 2-1
FIGURE 2-2
FIGURE 2-3

FIGURE 2-1

```
                1                                                   50
                                                                    ⌐
hp25a           MNTSHLLALL LPKSPQGENR SKPLGTPYNF SEHCQDSVDV MVFIVTSYSI
human Y1        MN.STLFSQV ENHSVHSNFS EKNAQLLAFE NDDCHLPLAM IFTLALAYGA
rat Y1          MN.STLFSRV ENYSVHYNVS E.NSPFLAFE NDDCHLPLAV IFTLALAYGA
mouse Y1        MN.STLFSKV ENHSIHYNAS E.NSPLLAFE NDDCHLPLAV IFTLALAYGA

               51                                                  100
                — I ——————————⌐                    ⌐———————— II ———
hp25a           ETVVGVLGNL CLMCVTVRQK EKANVTNLLI ANLAFSDFLM CLLCQPLTAV
human Y1        VIILGVSGNL ALIIIILKQK EMRNVTNILI VNLSFSDLLV AIMCLPFTFV
rat Y1          VIILGVSGNL ALIIIILKQK EMRNVTNILI VNLSFSDLLV AVMCLPFTFV
mouse Y1        VIILGVSGNL ALIIIILKQK EMRNVTNILI VNLSFSDLLV AVMCLPFTFV

               101                                                 150
                ⌐——————————————⌐———— III ————⌐
hp25a           YTIMDYWIFG ETLCKMSAFI QCMSVTVSIL SLVLVALERH QLIINPTGWK
human Y1        YTLMDHWVFG EAMCKLNPFV QCVSITVSIF SLVLIAVERH QLIINPRGWR
rat Y1          YTLMDHWVFG ETMCKLNPFV QCVSITVSIF SLVLIAVERH QLIINPRGWR
mouse Y1        YTLMDHWVFG ETMCKLNPFV QCVSITVSIF SLVLIAVERH QLIINPRGWR

               151                                                 200
                ⌐———————————— IV ————⌐
hp25a           PSISQAYLGI VLIWVIACVL SLPFLANSIL ENVFHKNHSK ALEFLADKVV
human Y1        PNNRHAYVGI AVIWVLAVAS SLPFLIYQVM TDEPFQNVT. .LDAYKDKYV
rat Y1          PNNRHAYIGI TVIWVLAVAS SLPFVIYQIL TDEPFQNVS. .LAAFKDKYV
mouse Y1        PNNRHAYIGI TVIWVLAVAS SLPFVIYQIL TDEPFQNVS. .LAAFKDKYV
```

FIGURE 2-2

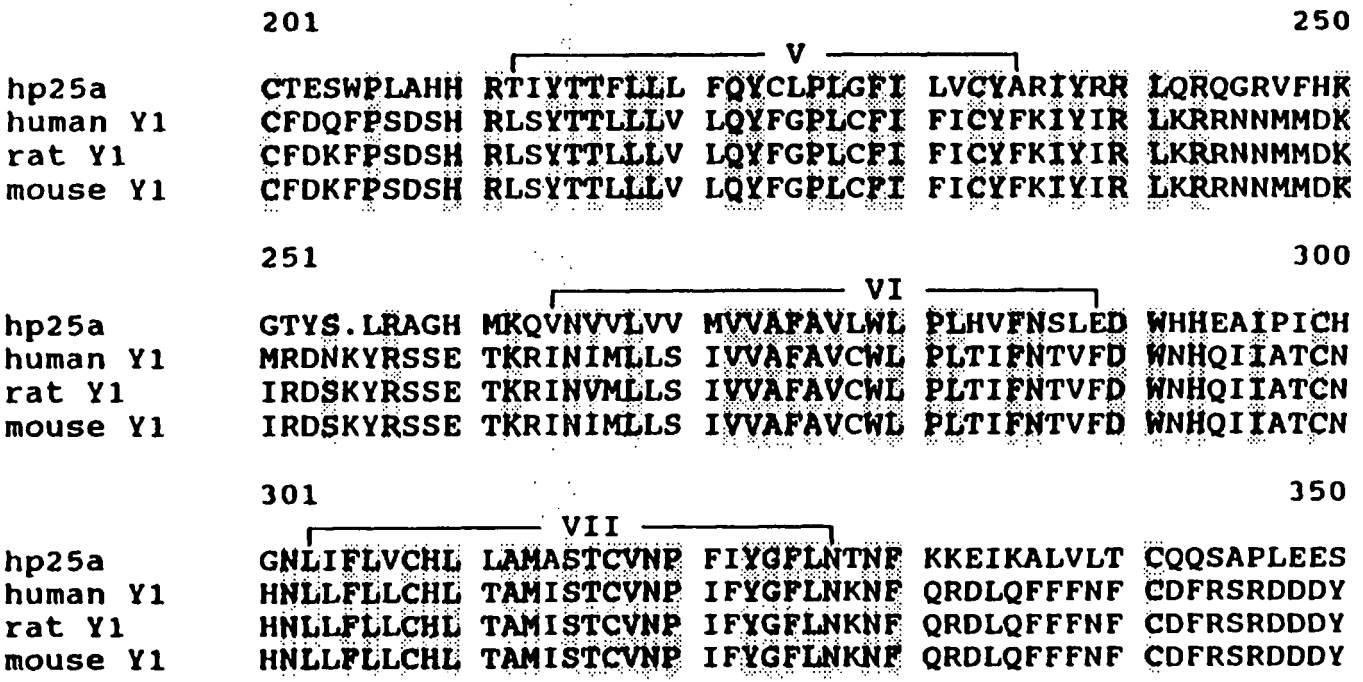

201
250
V
hp25a CTESWPLAHH RTIYTTFLLL FQYCLPLGFI LVCYARIYRR LQRQGRVFHK
human Y1 CFDQFPSDSH RLSYTTLLLV LQYFGPLCFI FICYFKIYIR LKRRNNMMDK
rat Y1 CFDKFPSDSH RLSYTTLLLV LQYFGPLCFI FICYFKIYIR LKRRNNMMDK
mouse Y1 CFDKFPSDSH RLSYTTLLLV LQYFGPLCFI FICYFKIYIR LKRRNNMMDK
251
300
VI
hp25a GTYS.LRAGH MKQVNVVLVV MVVAFAVLWL PLHVFNSLED WHHEAIPICH
human Y1 MRDNKYRSSE TKRINIMLLS IVVAFAVCWL PLTIFNTVFD WNHQIIATCN
rat Y1 IRDSKYRSSE TKRINVMLLS IVVAFAVCWL PLTIFNTVFD WNHQIIATCN
mouse Y1 IRDSKYRSSE TKRINIMLLS IVVAFAVCWL PLTIFNTVFD WNHQIIATCN
301
350
VII
hp25a GNLIFLVCHL LAMASTCVNP FIYGFLNTNF KKEIKALVLT CQQSAPLEES
human Y1 HNLLFLLCHL TAMISTCVNP IFYGFLNKNF QRDLQFFFNF CDFRSRDDDY
rat Y1 HNLLFLLCHL TAMISTCVNP IFYGFLNKNF QRDLQFFFNF CDFRSRDDDY
mouse Y1 HNLLFLLCHL TAMISTCVNP IFYGFLNKNF QRDLQFFFNF CDFRSRDDDY

FIGURE 2-3

```
                                                    388
hp25a       351 EHLPLSTVHT EVSKGSLRLS GRSNPI*... ........
human Y1        ETIAMSTMHT DVSKTSLKQA SPVAFKKINN NDDNEKI*
rat Y1          ETIAMSTMHT DVSKTSLKQA SPVAFKKISM N.DNEKI*
mouse Y1        ETIAMSTMHT DVSKTSLKQA SPVAFKKISM N.DNEKV*
```

FIGURE 3-1

FIGURE 3-1
FIGURE 3-2
FIGURE 3-3
FIGURE 3-4

```
   -170                -150                -130
        .         .         .         .         .         .
ATAGCTCTCAAGCCATAAGATATAAGTAGCTAAGAATTGTCTCCCTCTCCCTGTCCCTTG

   -110                 -90                 -70
        .         .         .         .         .         .
TTCTTACCTGGTTCCATTTTACATGCCTGGACCTTTGAGTTCCATTTGTTTGTTTTGCAG

     -50                 -30                 -10
        .         .         .         .         .         .
GCTACACTCAGAAGTGGGCCCTTTAGTCTTGAAGTTCCTGGTCTTCTCACACCCACCATG
                                                        M

      10                  30                  50
        .         .         .         .         .         .
AATACCTCTCATCTCATGGCCTCCCTTTCTCCGGCATTCCTACAAGGTAAGAATGGGACC
N  T  S  H  L  M  A  S  L  S  P  A  F  L  Q  G  K  N  G  T

      70                  90                 110
        .         .         .         .         .         .
AACCCACTGGATTCCCTCTATAATCTCTCTGACGGCTGCCAGGATTCGGCAGATCTGTTG
N  P  L  D  S  L  Y  N  L  S  D  G  C  Q  D  S  A  D  L  L

     130                 150                 170
        .         .         .         .         .         .
GCCTTCATCATCACCACCTACAGCGTTGAGACCGTCTTGGGGGTCCTAGGAAACCTCTGC
A  F  I  I  T  T  Y  S  V  E  T  V  L  G  V  L  G  N  L  C

     190                 210                 230
        .         .         .         .         .         .
TTGATATTTGTGACCACAAGGCAAAAGGAAAAGTCCAATGTGACCAACCTACTCATTGCC
L  I  F  V  T  T  R  Q  K  E  K  S  N  V  T  N  L  L  I  A
```

FIGURE 3-2

```
     250                 270                 290
      .         .         .         .         .         .
AACCTGGCCTTCTCTGACTTCCTCATGTGTCTCATCTGCCAGCCGCTCACGGTCACCTAC
N  L  A  F  S  D  F  L  M  C  L  I  C  Q  P  L  T  V  T  Y

     310                 330                 350
      .         .         .         .         .         .
ACCATCATGGACTACTGGATCTTCGGCGAAGTCCTTTGCAAGATGTTAACGTTCATCCAG
T  I  M  D  Y  W  I  F  G  E  V  L  C  K  M  L  T  F  I  Q

     370                 390                 410
      .         .         .         .         .         .
TGTATGTCGGTGACAGTCTCCATCCTCTCACTGGTCCTTGTGGCCCTGGAGAGGCACCAG
C  M  S  V  T  V  S  I  L  S  L  V  L  V  A  L  E  R  H  Q

     430                 450                 470
      .         .         .         .         .         .
CTCATTATCAACCCGACTGGCTGGAAACCCAGCATTTCCCAGGCCTACCTGGGGATTGTG
L  I  I  N  P  T  G  W  K  P  S  I  S  Q  A  Y  L  G  I  V

     490                 510                 530
      .         .         .         .         .         .
GTCATCTGGTTCATTTCTTGTTTCCTCTCCTTGCCCTTCCTGGCCAATAGCATCCTGAAC
V  I  W  F  I  S  C  F  L  S  L  P  F  L  A  N  S  I  L  N

     550                 570                 590
      .         .         .         .         .         .
GACCTCTTCCACTACAACCACTCTAAGGTTGTGGAGTTTCTGGAAGACAAGGTTGTCTGC
D  L  F  H  Y  N  H  S  K  V  V  E  F  L  E  D  K  V  V  C

     610                 630                 650
      .         .         .         .         .         .
TTTGTGTCCTGGTCCTCGGATCACCACCGCCTCATCTACACCACCTTTCTGCTGCTCTTC
F  V  S  W  S  S  D  H  H  R  L  I  Y  T  T  F  L  L  L  F
```

FIGURE 3-3

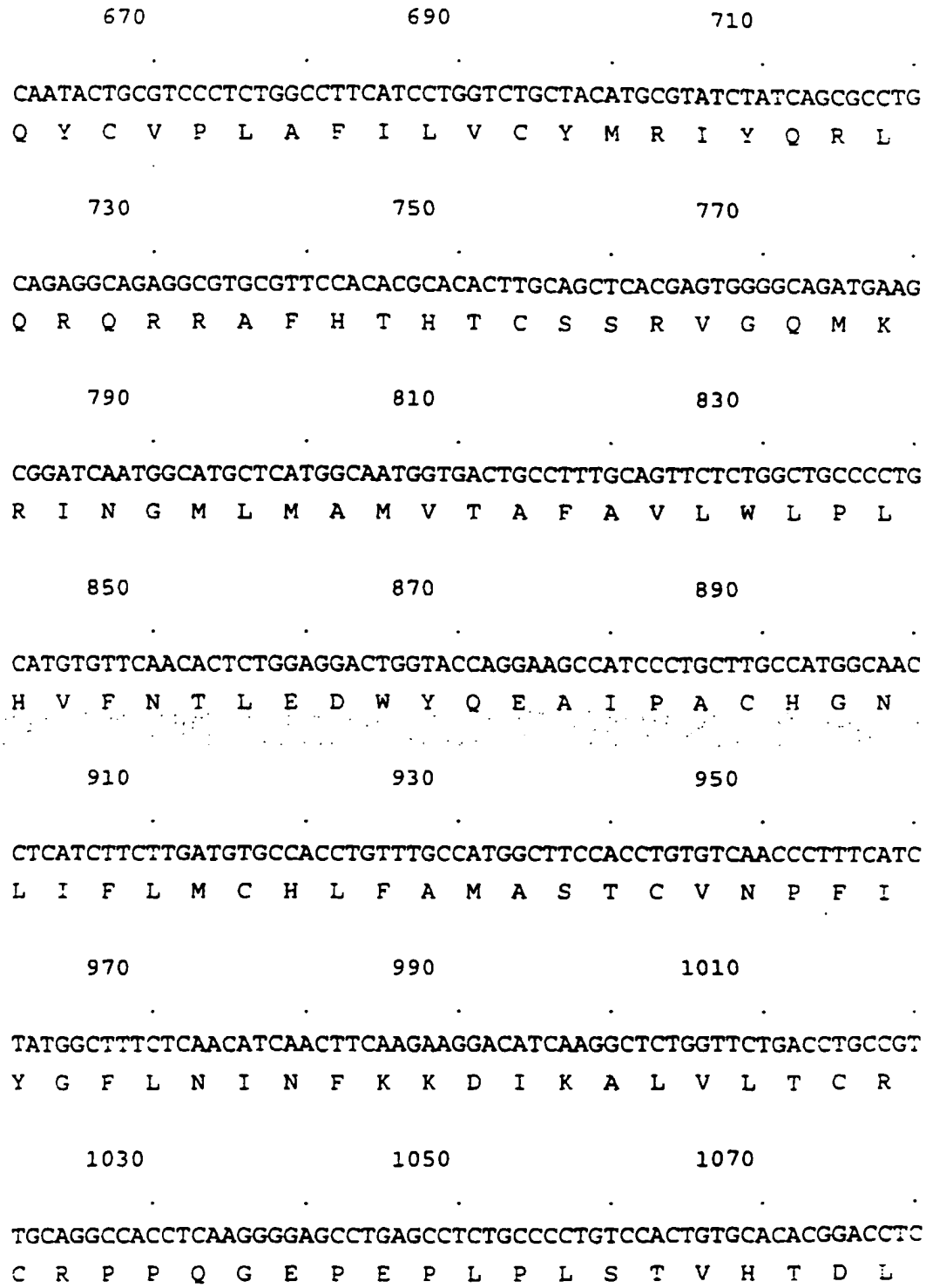

670
690
710
CAATACTGCGTCCCTCTGGCCTTCATCCTGGTCTGCTACATGCGTATCTATCAGCGCCTG
Q Y C V P L A F I L V C Y M R I Y Q R L
730
750
770
CAGAGGCAGAGGCGTGCGTTCCACACGCACACTTGCAGCTCACGAGTGGGGCAGATGAAG
Q R Q R R A F H T H T C S S R V G Q M K
790
810
830
CGGATCAATGGCATGCTCATGGCAATGGTGACTGCCTTTGCAGTTCTCTGGCTGCCCCTG
R I N G M L M A M V T A F A V L W L P L
850
870
890
CATGTGTTCAACACTCTGGAGGACTGGTACCAGGAAGCCATCCCTGCTTGCCATGGCAAC
H V F N T L E D W Y Q E A I P A C H G N
910
930
950
CTCATCTTCTTGATGTGCCACCTGTTTGCCATGGCTTCCACCTGTGTCAACCCTTTCATC
L I F L M C H L F A M A S T C V N P F I
970
990
1010
TATGGCTTTCTCAACATCAACTTCAAGAAGGACATCAAGGCTCTGGTTCTGACCTGCCGT
Y G F L N I N F K K D I K A L V L T C R
1030
1050
1070
TGCAGGCCACCTCAAGGGGAGCCTGAGCCTCTGCCCCTGTCCACTGTGCACACGGACCTC
C R P P Q G E P E P L P L S T V H T D L

FIGURE 3-4

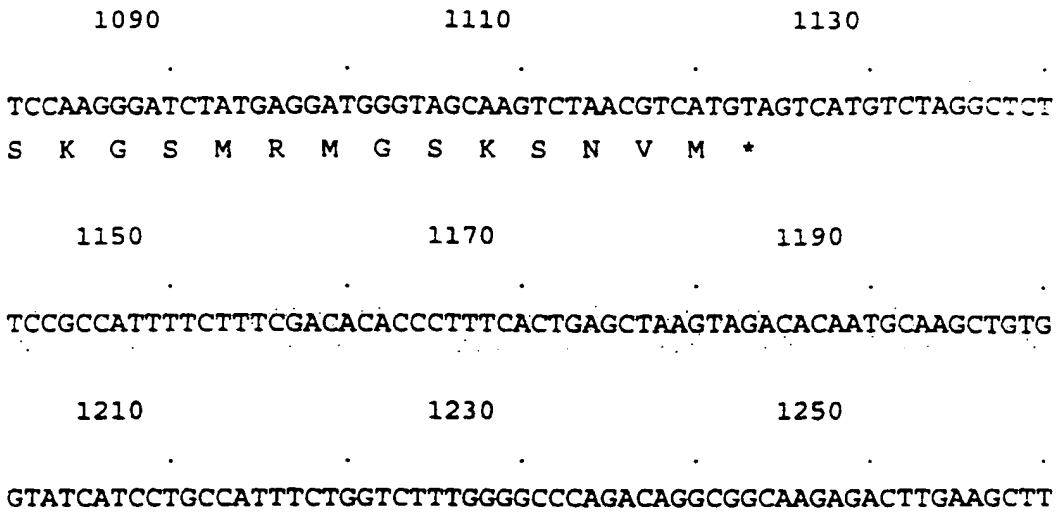

1090
1110
1130
TCCAAGGGATCTATGAGGATGGGTAGCAAGTCTAACGTCATGTAGTCATGTCTAGGCTCT
S K G S M R M G S K S N V M *
1150
1170
1190
TCCGCCATTTTCTTTCGACACACCCTTTCACTGAGCTAAGTAGACACAATGCAAGCTGTG
1210
1230
1250
GTATCATCCTGCCATTTCTGGTCTTTGGGGCCCAGACAGGCGGCAAGAGACTTGAAGCTT

FIGURE 4

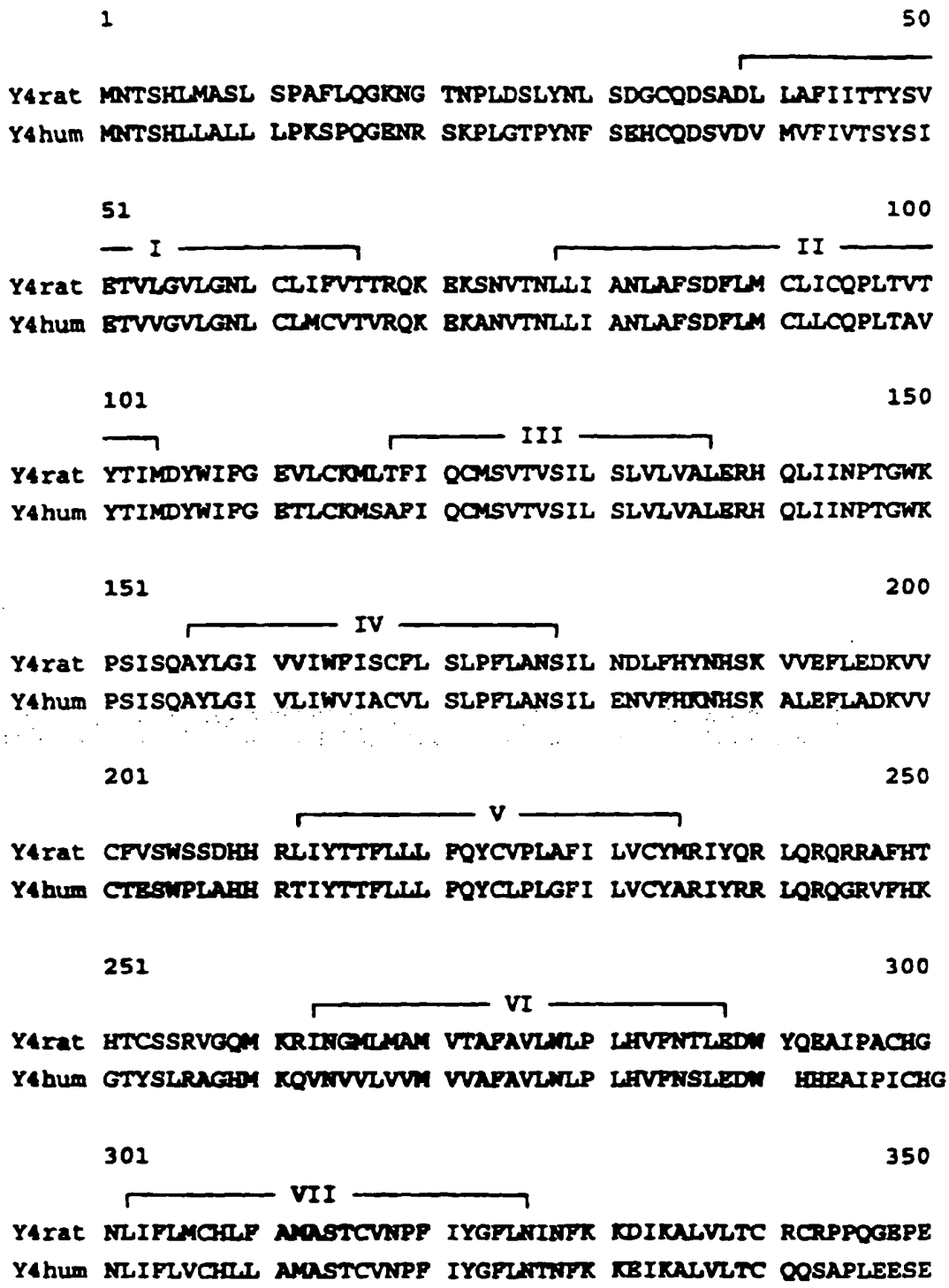

1
50
Y4rat MNTSHLMASL SPAFLQGKNG TNPLDSLYNL SDGCQDSADL LAFIITTYSV
Y4hum MNTSHLLALL LPKSPQGENR SKPLGTPYNF SEHCQDSVDV MVFIVTSYSI
51
100
I
II
Y4rat ETVLGVLGNL CLIFVTTRQK EKSNVTNLLI ANLAFSDFLM CLICQPLTVT
Y4hum ETVVGVLGNL CLMCVTVRQK EKANVTNLLI ANLAFSDFLM CLLCQPLTAV
101
150
III
Y4rat YTIMDYWIPG EVLCKMLTFI QCMSVTVSIL SLVLVALERH QLIINPTGWK
Y4hum YTIMDYWIPG ETLCKMSAFI QCMSVTVSIL SLVLVALERH QLIINPTGWK
151
200
IV
Y4rat PSISQAYLGI VVIWFISCFL SLPFLANSIL NDLFHYNHSK VVEFLEDKVV
Y4hum PSISQAYLGI VLIWVIACVL SLPFLANSIL ENVFHKNHSK ALEFLADKVV
201
250
V
Y4rat CFVSWSSDHH RLIYTTFLLL FQYCVPLAFI LVCYMRIYQR LQRQRRAFHT
Y4hum CTESWPLAHH RTIYTTFLLL FQYCLPLGFI LVCYARIYRR LQRQGRVFHK
251
300
VI
Y4rat HTCSSRVGQM KRINGMLMAM VTAFAVLWLP LHVFNTLEDW YQEAIPACHG
Y4hum GTYSLRAGHM KQVNVVLVVM VVAFAVLWLP LHVFNSLEDW HHEAIPICHG
301
350
VII
Y4rat NLIFLMCHLF AMASTCVNPF IYGFLNINFK KDIKALVLTC RCRPPQGEPE
Y4hum NLIFLVCHLL AMASTCVNPF IYGFLNTNFK KEIKALVLTC QQSAPLEESE

FIGURE 5

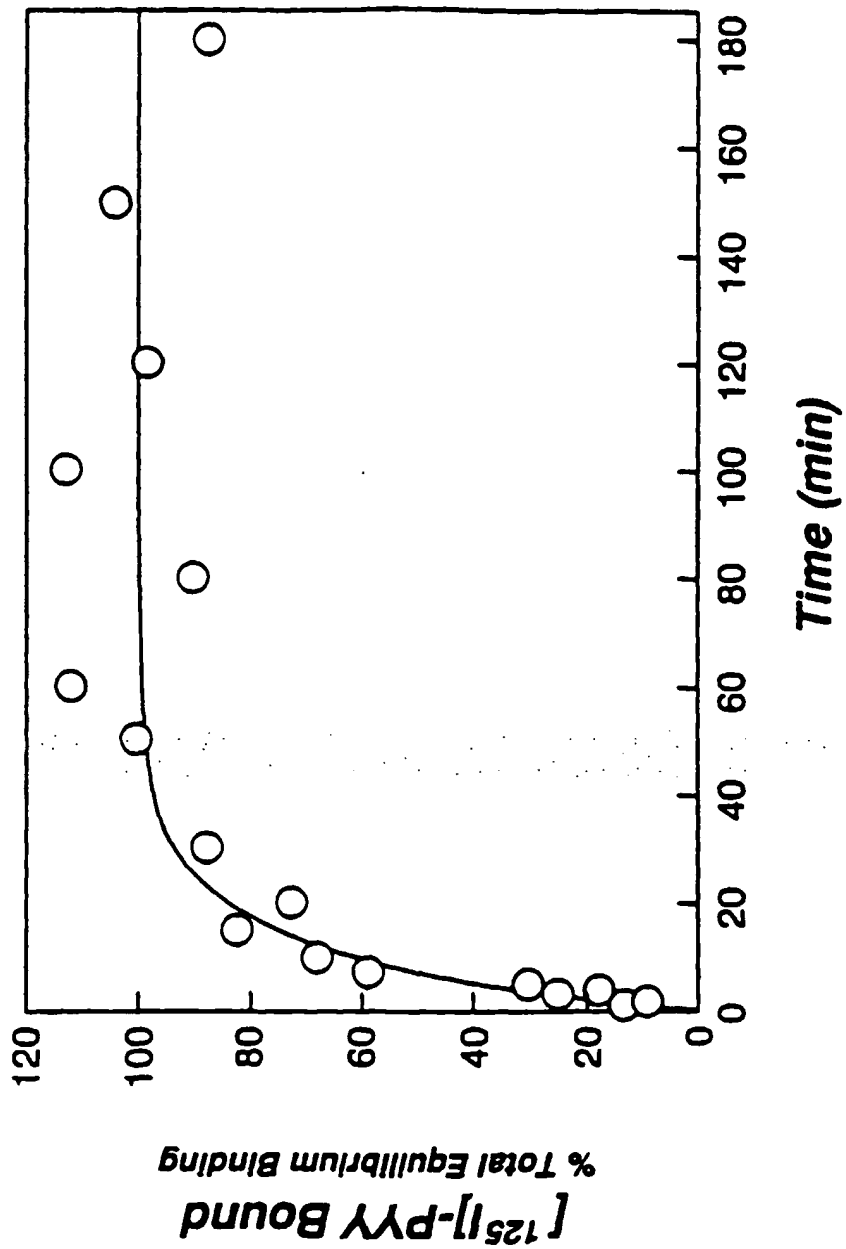

[125I]-PYY Bound
% Total Equilibrium Binding
120
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
180
Time (min)

FIGURE 6A

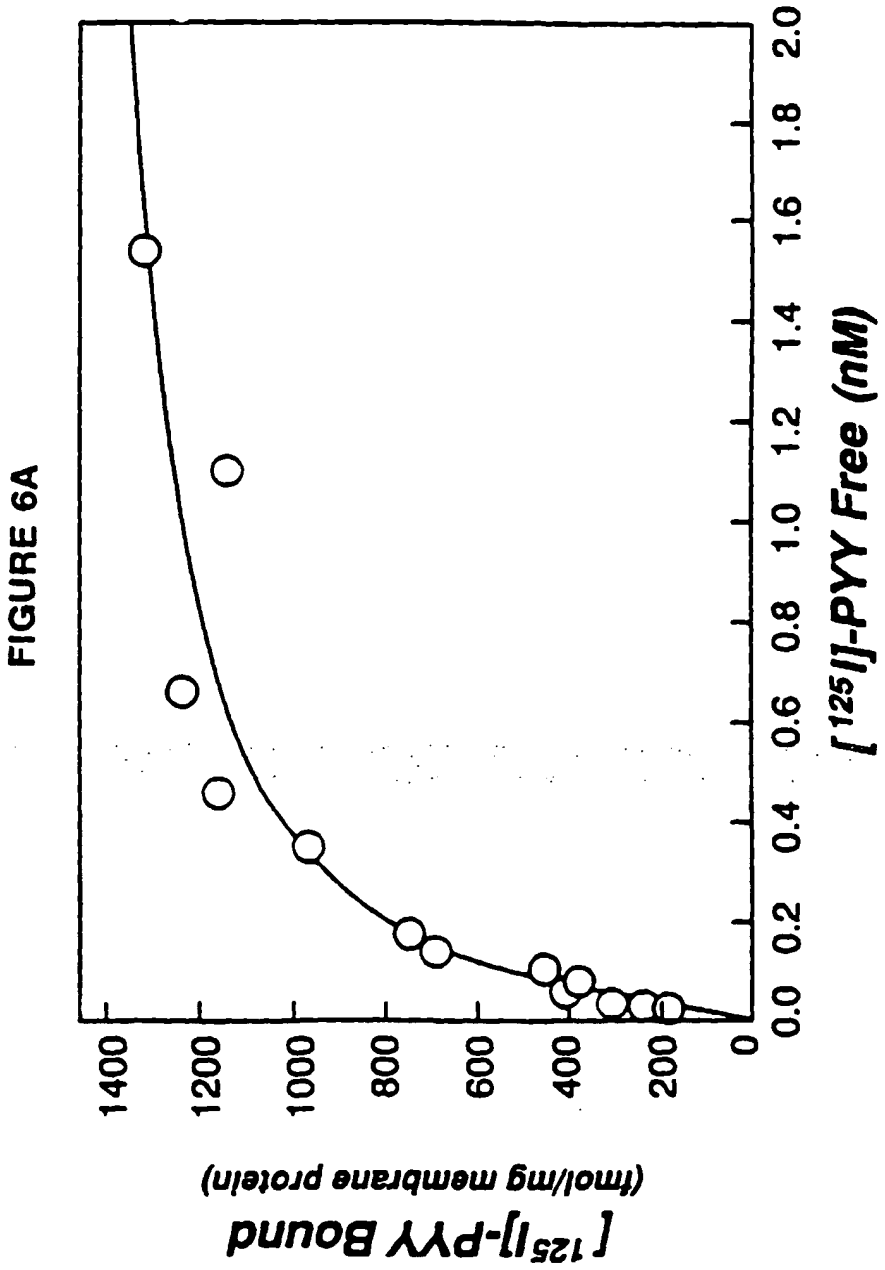

[125I]-PYY Bound
(fmol/mg membrane protein)
1400
1200
1000
800
600
400
200
0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
[125I]-PYY Free (nM)

FIGURE 6B

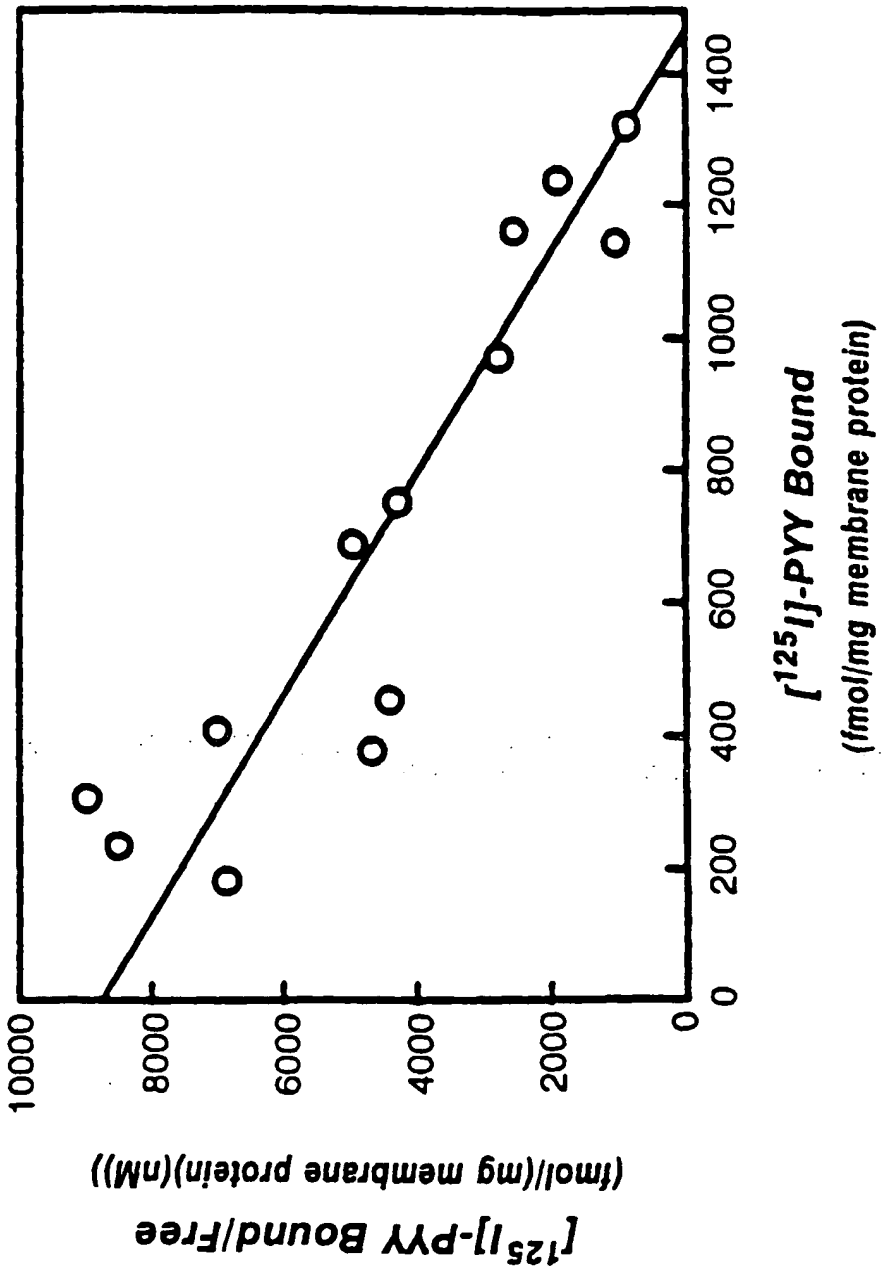

[125I]-PYY Bound/Free
(fmol/(mg membrane protein)(nM))
10000
8000
6000
4000
2000
0
0
200
400
600
800
1000
1200
1400
[125I]-PYY Bound
(fmol/mg membrane protein)

FIGURE 7

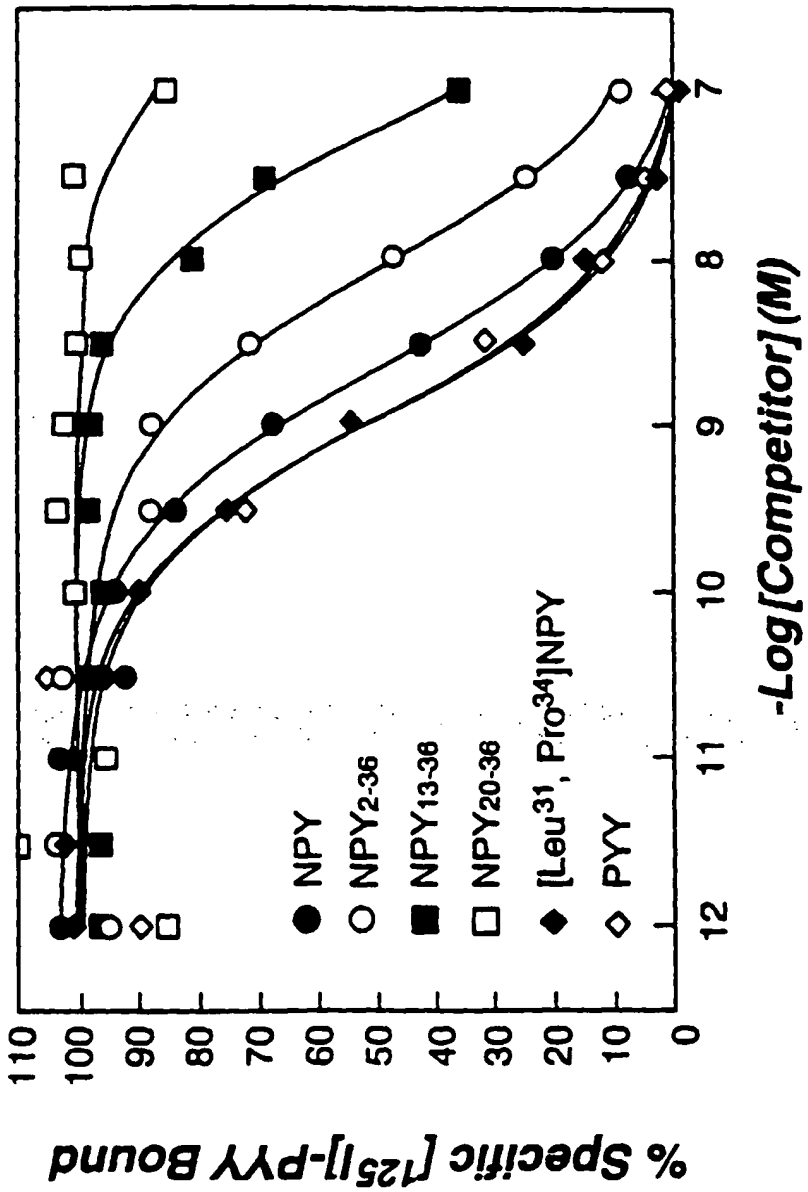

% Specific [125I]-PYY Bound
110
100
90
80
70
60
50
40
30
20
10
0
12
11
10
9
8
7
-Log[Competitor](M)
NPY
NPY2-36
NPY13-36
NPY20-36
[Leu31, Pro34]NPY
PYY

FIGURE 8

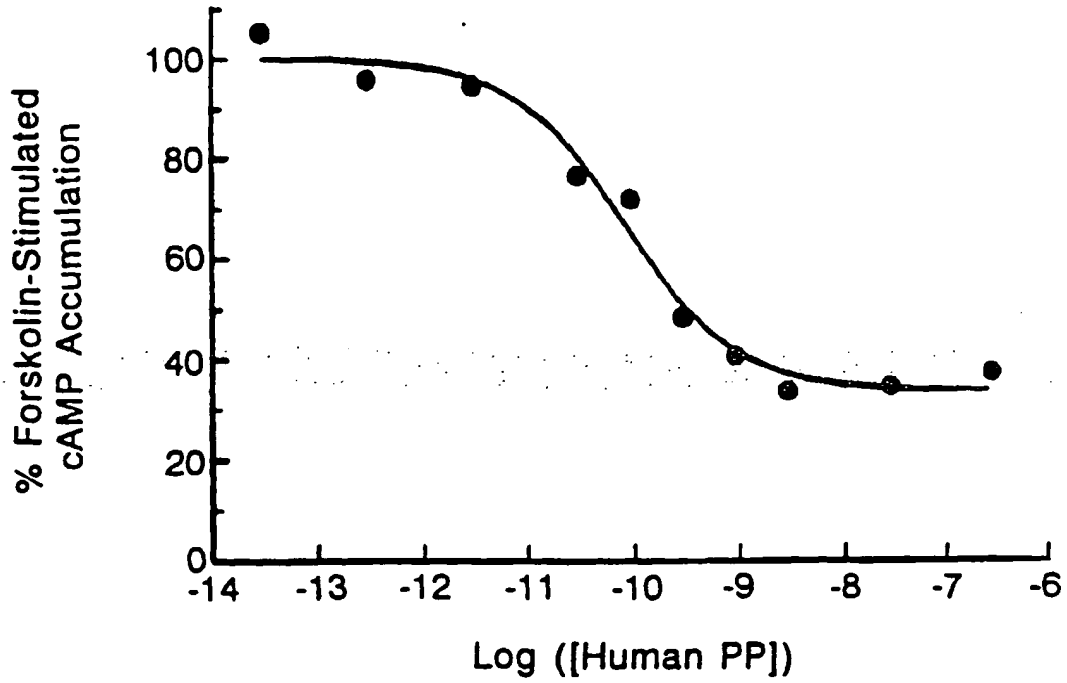

% Forskolin-Stimulated cAMP Accumulation
100
80
60
40
20
0
-14
-13
-12
-11
-10
-9
-8
-7
-6
Log ([Human PP])

FIGURE 9A

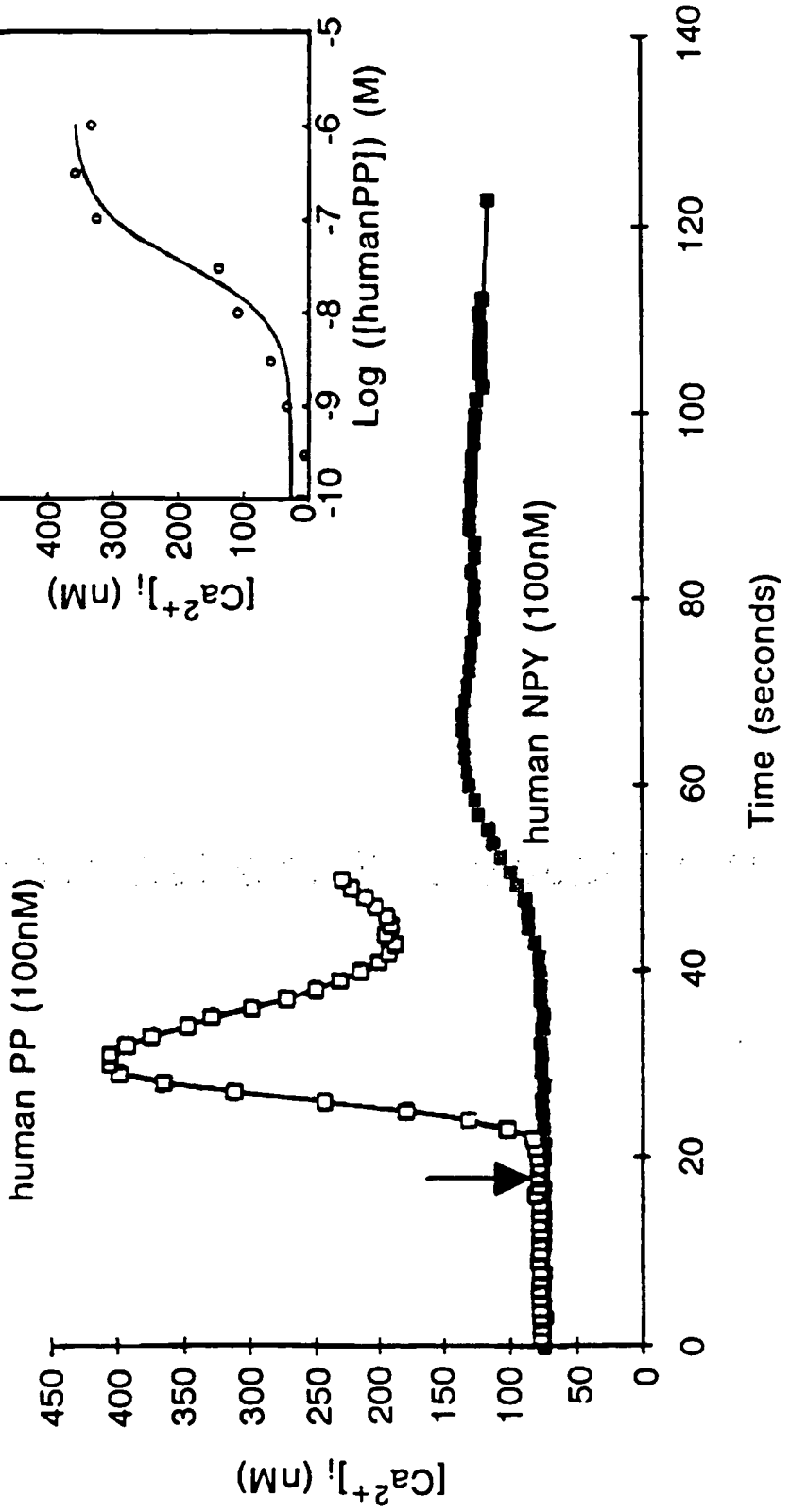


FIGURE 9B